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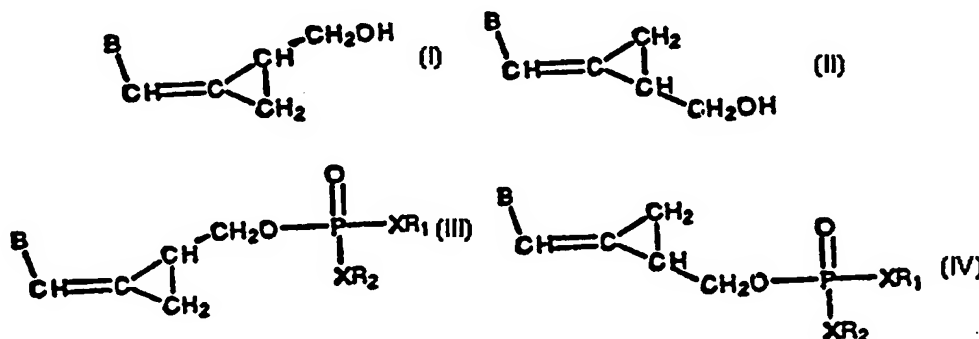
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(54) Title: 2-HYDROXYMETHYLCYCLOPROPYLI- DENEMETHYLPURINES AND -PYRIMIDINES AS ANTIVIRAL AGENTS



(57) Abstract

Compounds which are active against viruses have Formulas (I), (II), (III) and (IV), wherein B is a purine or pyrimidine heterocyclic ring and is preferably selected from the group consisting of 6-aminopurine (adenine), 2,6-diaminopurine, 2-amino-6-cyclopropylaminopurine, 6-hydroxypurine (hypoxanthine), 2-amino-6-halo substituted purines, 2-amino-6-alkoxy substituted purines, 2-amino-6-hydroxypurine (guanine), 3-deazapurines, 7-deazapurines, 8-azapurines, cytosine, 5-halo substituted cytosines, 5-alkyl substituted cytosines, thymine, uracil and 6-azapyrimidines; X is O; and R₁ and R₂ are alkyl or aryl groups. The R₁X and/or R₂X can also be amino acid residues with X as NH.

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2-HYDROXYMETHYLCYCLOPROPYLIDENEMETHYLPURINES AND -PYRIMIDINES AS ANTIVIRAL AGENTS

SPONSORSHIP

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the invention.

FIELD OF THE INVENTION

The present invention relates generally to novel purine and pyrimidine
10 compounds which have antiviral activity and methods of making and using same.

BACKGROUND OF THE INVENTION

Viruses are the etiologic cause of many life-threatening human diseases. Of
special importance are herpes viruses such as herpes simplex 1 (HSV-1), herpes
simplex 2 (HSV-2), cytomegalovirus (CMV), Epstein-Barr virus (EBV), varicella
15 zoster virus (VZV) and human herpes virus 6 (HHV 6) which are associated with
many common viral illnesses. The HSV-1 and HSV-2 infections are characterized
by cold sores of skin, mouth or genital region. After primary infection the virus is
harbored in neural cells and can reappear later in the life of a patient. Human CMV
(HCMV) infection is a life-long affliction which can result in morbidity and mortality.
20 These pathologies include microcephaly, hepatosplenomegaly, jaundice,
encephalitis, infections of the newborn infants or fetuses in utero, and infections of
immunocompromised hosts. The HCMV infection is responsible for retinitis, gastritis
and pneumonitis in AIDS patients and HCMV-induced pneumonias or hepatitis are
frequent and serious complications of bone marrow transplants. EBV causes
25 infectious mononucleosis and it is considered as the etiologic agent of
nasopharyngeal cancer, immunoblastic lymphoma, Burkitt's lymphoma and hairy
leukoplakia. VZV causes chicken pox and shingles. Although in children the chicken
pox is usually a non-fatal disease, the recurrent form of this infection, shingles, may
in advanced stage lead to paralysis, convulsions and ultimately death. Again, in
30 immunocompromised patients the infection with VZV is a serious complication.
Human herpes virus 6 (HHV-6) which is commonly associated with children's rash
was also identified in acquired immunodeficiency syndrome (AIDS) patients and it
may be a cofactor in the pathogenesis of AIDS in hosts infected with human
immunodeficiency virus (HIV). Levine, A.J., Viruses, Ch. 4, W. H. Freeman & Co.,
35 New York, pp. 67-85 (1992); Human Herpesvirus Infections, Raven Press, New York
(1986).

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HIV is the underlying cause of AIDS, a world-wide epidemic with fatal consequences. According to the World Health Organization, over 4.5 million AIDS cases were recorded by late 1994 and 19.5 million people had been infected with HIV. It is estimated that by the year 2000, 30 to 40 million will have been infected with HIV with 10 million cases of full-blown AIDS. Estimates of Global AIDS Policy Coalition are considerably higher - up to 110 million HIV infections and 25 million AIDS cases. The Relationship between the Human Immunodeficiency Virus and the Acquired Immunodeficiency Syndrome, The National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, pp. 1-3 (1995).

Various alkyl derivatives of purines and pyrimidines and analogues thereof have been found to exhibit antiviral activity. Notably, acyclovir (Zovirax) and ganciclovir (Cytovene) belonging to this group of compounds are approved drugs for infections caused by HSV-1, HSV-2, VZV and HCMV. Acyclovir Therapy for Herpesvirus Infections (Baker, Ed.), M. Dekker, New York, (1990); Ganciclovir Therapy for Cytomegalovirus Infection (Spector, S. S., Ed.), M. Dekker, New York (1991). A considerable effort went into design, synthesis and biological investigation of analogues of these drugs as well as in development of new antiviral agents. Larsson, A., et al., *Antimicrob. Agents & Chemother.* 30:598-605 (1986); Ashton, W.T., et al., *J. Med. Chem.* 31:2304-2315 (1988).

Current drugs for AIDS include AZT (zidovudine, Retrovir), ddI (didanosine, Videx), ddC (zalcitabine, Hivid) and d4T (stavudine, Zerit). De Clercq, E., *J. Med. Chem.* 38:2491-2517 (1995). Allenic nucleoside analogues such as adenallene and cytallene are examples of anti-HIV agents containing an unsaturated alkyl group. U.S. Patent No. 4,935,427; Zemlicka, J., Allenols Derived from Nucleic Acid Bases - a New Class of Anti-HIV Agents: Chemistry and Biological Activity in Nucleosides and Nucleotides as Antitumor and Antiviral Agents (Chu, C. K.; Baker, D. C., Eds.), Plenum Press, New York, pp. 73-100 (1993).

It would thus be desirable to provide novel compounds which are active against viruses, including HCMV, HSV-1, HSV-2, HHV-6, HIV, hepatitis B virus (HBV), and other mammalian viruses.

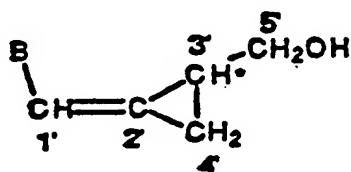
SUMMARY OF THE INVENTION

The present invention provides novel 2-hydroxymethylcyclopropylidenemethyl-derivatives of heterocyclic compounds, prodrugs and pharmacologically acceptable acid salts thereof. These compounds have been found to be useful antiviral agents

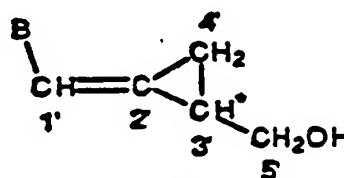
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and are effective against HCMV, HSV-1, HSV-2, HHV-6, HIV and HBV, as well as against other mammalian viruses.

The compounds of the present invention have the following Formulas:



Formula 1



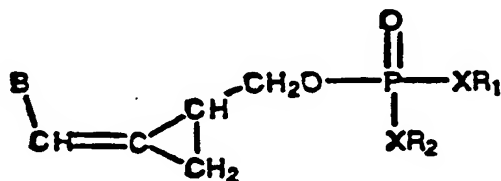
Formula 2

wherein

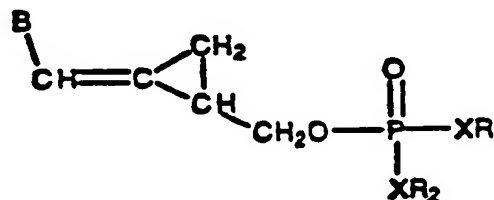
- 5 B is a heterocyclic ring derived from a purine or pyrimidine moiety. In a preferred embodiment, the purine moieties include 6-aminopurine (adenine), 2,6-diaminopurine, 2-amino-6-cyclopropylaminopurine, 6-hydroxypurine (hypoxanthine), 2-amino-6-halo substituted purines, such as 2-amino-6-chloropurine, 2-amino-6-alkoxy substituted purines, such as 2-amino-6-methoxypurine, 2-amino-6-
10 hydroxypurine (guanine), 3-deazapurines, 7-deazapurines, 8-azapurines, cytosine, 5-halo substituted cytosines, 5-alkyl substituted cytosines, thymine, uracil and 6-azapyrimidines.

Prodrugs of the antiviral nucleoside analogues of the present invention include lipophilic phosphate esters or amidates capable of penetrating the cell
15 membrane. Those skilled in the art will appreciate that the aim of prodrugs is to provide effective therapeutic agents for resistant strains of viruses (McGuigan, C., et al., *J. Med. Chem.* 36:1048-1052 (1993)) or activate inactive analogues. Franchetti, P., et al., *J. Med. Chem.* 37:3534-3541 (1994).

The compounds of the present invention therefore also include prodrugs of
20 the novel compounds, wherein the prodrugs have the following Formulas:



Formula 3



Formula 4

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wherein

B is a heterocyclic ring as defined above for Formulas 1 and 2;

X is O; and

R₁ and R₂ are alkyl or aryl. The R₁X or R₂X may also be amino acid residues
5 with X as NH.

Compositions useful for treating viral infections, such as HCMV, HSV-1, HSV-2, HHV-6, HIV and HBV contain an effective amount of at least one compound of Formulas 1 to 4 or a pharmaceutically acceptable salt thereof.

The present invention also includes methods for synthesizing compounds of
10 Formulas 1 and 2 wherein B is a heterocyclic ring derived from purine or pyrimidine moiety such as 6-aminopurine (adenine), 2,6-diaminopurine, 2-amino-6-cyclopropylaminopurine, 6-hydroxypurine (hypoxanthine), 2-amino-6-halo substituted purines, such as 2-amino-6-chloropurine, 2-amino-6-alkoxy substituted purines, such as 2-amino-6-methoxypurine, 2-amino-6-hydroxypurine (guanine), 3- and 7-
15 deazapurines, such as 3- and 7-deazaadenine, 8-azapurines such as 8-azaadenine, cytosine, 5-halocytosine (wherein halo is bromo, chloro, iodo or fluoro) and related alkyl derivatives containing a saturated or unsaturated alkyl group at the 5-position, thymine, uracil, 6-azapyrimidines such as 6-azacytosine, wherein the alkyl side-chain attached to the heterocyclic ring is a 2-hydroxymethylcyclopropylidenemethane
20 moiety.

The present invention also provides methods for synthesizing prodrugs of the compounds as set forth in Formulas 3 and 4.

Additional objects, advantages, and features of the present invention will become apparent from the following description, taken in conjunction with the
25 accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The various advantages of the present invention will become apparent to one skilled in the art by reading the following specification and by referencing the following drawings in which:

30 Figure 1 shows the synthesis of the mixture of *cis*-ethyl 2-bromo-2-bromomethylcyclopropane 1-carboxylate (6) and *trans*-ethyl 2-bromo-2-bromomethylcyclopropane 1-carboxylate (7);

Figure 2 shows the synthesis of purine and pyrimidine 2-hydroxymethylcyclopropylidenemethylpurines and -pyrimidines of Formulas 1 and 2;

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Figure 3 shows the method for separation of *syn*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)adenine (1, B = adenin-N⁹-yl) and *anti*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)adenine (2, B = adenin-N⁹-yl);

Figure 4 shows the hydrolysis of *syn*-2-amino-6-chloro-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine (1, B = 2-amino-6-chloropurine) and *anti*-2-amino-6-chloro-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine (1, B = 2-amino-6-chloropurine) to *syn*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)guanine (1, B = guanine) and *anti*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)guanine (1, B = guanine). Figure 4 also shows the synthesis of *syn*-2,6-diamino-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine (1, B = 2,6-diaminopurine) and *syn*-2-amino-6-cyclopropylamino-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine (1, B = 2-amino-6-cyclopropylaminopurine) from *syn*-2-amino-6-chloro-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine (1, B = 2-amino-6-chloro- purine);

Figure 5 shows the method for separation of *syn*-N¹-(2-hydroxymethylcyclopropylidenemethyl)cytosine (1, B = cytosin-N¹-yl) and *trans*-N¹-(2-hydroxymethylcyclopropylidenemethyl)cytosine (2, B = cytosin-N¹-yl);

Figure 6 shows the synthesis of methyl phenylphosphoro-L-alaninate of *syn*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)adenine (3, B = adenin-N⁹-yl); and

Figure 7 shows the synthesis of methyl phenylphosphoro-L-alaninate of *anti*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)adenine (4, B = adenin-N⁹-yl).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The compounds of the present invention which have been found to be effective against herpes viruses, human immunodeficiency virus, and hepatitis B virus, are compounds of Formulas 1 to 4, wherein B represents a heterocyclic ring derived from purine or pyrimidine moiety such as 6-aminopurine (adenine), 2,6-diaminopurine, 2-amino-6-cyclopropylaminopurine, 6-hydroxypurine (hypoxanthine), 2-amino-6-halo substituted purines, such as 2-amino-6-chloropurine, 2-amino-6-alkoxy substituted purines, such as 2-amino-6-methoxypurine, 2-amino-6-hydroxypurine (guanine), 3- and 7-deazapurines, such as 3- and 7-deazaadenine, 8-azapurines such as 8-azaadenine, cytosine, 5-halocytosine (wherein halo is bromo, chloro, iodo, or fluoro) and related alkyl derivatives containing a saturated or unsaturated alkyl group at the 5-position, thymine, uracil or 6-azapyrimidine, wherein the alkyl side-chain attached to the heterocyclic ring is a 2-hydroxymethylcyclopropylidenemethane moiety. The R₁, R₂ in Formulas 3 and 4 are

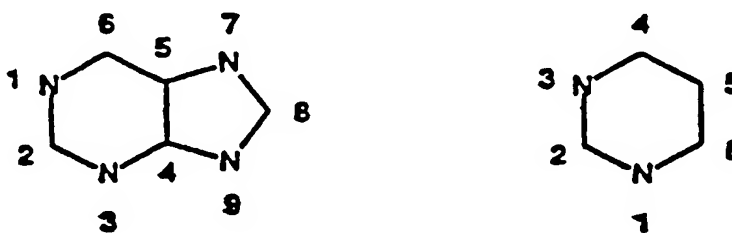
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alkyl or aryl groups, X is O and R₁X and R₂X can also be amino acid residues with X as NH.

The preferred compounds of the present invention are *syn*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)adenine (synadenol), wherein in Formula 1, B is adenin-N⁹-yl, *syn*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)guanine (synguanol), wherein B is guanin-N⁹-yl, *syn*-N¹-(2-hydroxymethylcyclopropylidenemethyl)cytosine (syncytol) wherein in Formula 1, B is cytosin-N¹-yl, *syn*-2,6-diamino-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine wherein Formula 1, B is 2,6-diaminopurine, *syn*-2-amino-6-cyclopropylamino-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine wherein in Formula 1, B is 2-amino-6-cyclopropylaminopurin-N⁹-yl and *syn*-2-amino-6-chloro-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine wherein in Formula 1, B is 2-amino-6-chloropurin-N⁹-yl.

The preferred compounds of the present invention are also methyl phenyl-phosphoro-L-alaninate of *syn*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)adenine (3, B = adenin-N⁹-yl) and *anti*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)adenine (4, B is adenin-N⁹-yl).

The nomenclature of the compounds of the present invention follows standard conventions. The numbering of the cyclopropylidenemethane moiety attached to the heterocyclic ring B is shown in Formulas 1 and 2. The purine and pyrimidine rings are numbered as indicated below:



It is appreciated that heterocyclic rings containing hydroxy and amino groups are tautomeric with the corresponding oxo and imino compounds.

The compounds described by Formulas 1 through 4 contain an asymmetric carbon atom marked in the Formulas 1 and 2 by an asterisk. Compounds of Formula 1 and 2 of the present invention are therefore racemic mixtures of two optical antipodes which may be resolved by conventional methods such as chromatography or fractional crystallization of suitable diastereoisomeric derivatives such as salts or esters with optically active acids (camphor 10-sulfonic acid, methoxyacetic acid,

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dibenzoyltartaric acid, 6-methoxy-2-naphthyl-2-propanoic acid, etc.), by an enantioselective enzymic synthesis of esters of one antipode such as acetates or butyrates or by an enantioselective enzymic hydrolysis of esters of compounds of Formulas 1 and 2 such as acetates or butyrates. The suitable enzymes include, but
5 are not limited to, lipases such as lipase AK, lipase P30 or esterases such as pig liver esterase. Racemic compounds containing adenine moiety may also be resolved by the action of adenosine deaminase.

Compounds 3 and 4 derived from racemic analogues 1 and 2 are mixtures of four diastereoisomers provided that R_1X is not the same as R_2X .

10 The syntheses of compounds of the present invention are summarized in Figures 1 to 7. Generally, alkyl *cis*- and *trans*-2-halo-2-halomethylcyclopropane 1-carboxylates can be used as suitable alkylating agents. Ethyl *cis*- and *trans*-2-chloro-2-chloromethylcyclopropane 1-carboxylates were previously described. Dyakonov, I.A., et al., *J. Gen. Chem. USSR* (English translation) 25:1435-1440 (1955). More
15 reactive *cis*- and *trans*-2-bromo-2-bromomethylcyclopropane 1-carboxylates 6 and 7, which are the preferred reagents, were prepared as follows: addition of ethyl diazoacetate to 2,3-dibromopropene (5) in the presence of suitable catalyst, e.g., dirhodium tetraacetate (Doyle, M.P., et al., *J. Org. Chem.* 46:5094-5102 (1981)) and organic solvent such as dichloromethane, afforded a mixture (1.5 : 1) of ethyl *cis* -
20 and *trans*-2-bromo-2-bromomethylcyclopropane 1-carboxylates (6 and 7) as described in Figure 1. The resultant mixture is then used for alkylation of heterocycles B-H (8, B = purine or pyrimidine moiety) in the presence of an appropriate base, e.g., potassium carbonate or sodium hydride in an organic solvent, e.g., N,N-dimethylformamide, to give a mixture of *cis*- and *trans*-1-bromo-2-
25 (carbethoxy)cyclopropylmethylpurines or -pyrimidines 9 and 10 in the ratio of 1.5 : 1 as described in Figure 2.

A mixture of bromoesters 9 and 10 was converted to a mixture of *syn*- and *anti*-2-carbethoxycyclopropylidenemethylpurines or -pyrimidines 11 and 12 (R = C_2H_5) using a strong base, e.g., potassium tert.-butoxide in an appropriate solvent
30 such as N,N-dimethylformamide as shown in Figure 2.

Alternately, the alkylation and elimination can be combined into a single step. Thus, the reaction of heterocycle B-H (8, B = purine or pyrimidine moiety) with ethyl 2-bromo-2-bromomethylcyclopropane 1-carboxylates (6 and 7) is performed with potassium carbonate in N,N-dimethylformamide at an elevated temperature to give
35 directly a mixture of the desired *syn*- and *anti*-2-carbethoxycyclopropylidenemethyl-

purines or -pyrimidines 11 and 12 ($R = C_2H_5$) as shown in Figure 2. A work up of the reaction mixture with methanol leads to transesterification to give methyl esters 11 and 12 ($R = CH_3$).

The reduction of carboethoxy or carbomethoxy group with a suitable reducing agent, e.g., diisobutylaluminum hydride and organic solvent such as tetrahydrofuran affords the compounds of the present invention, *syn*- and *anti*-2-hydroxymethylcyclopropylidenemethylpurines or -pyrimidines 1 and 2, as described in Figure 2. A mixture of compounds 1 and 2 is then separated by crystallization or chromatography on silica gel either directly or after a suitable derivatization.

For example, a mixture of *syn*- and *anti*- N^9 -(2-hydroxymethylcyclopropylidenemethyl)adenines 1 and 2 ($B = \text{adenin-}N^9\text{-yl}$) is converted to the corresponding N^6 -dimethylaminomethylene derivatives 1 and 2 ($B = N^6\text{-dimethylaminomethylene-adenin-}N^9\text{-yl}$) by reaction with *N,N*-dimethylformamide dimethyl acetal (Zemlicka, J., et al., *Collect. Czech. Chem. Commun.* 32:3159-3168 (1967)), in a suitable solvent such as *N,N*-dimethylformamide as described in Figure 3. Compounds 1 and 2 ($B = N^6\text{-dimethylaminomethyleneadenin-}N^9\text{-yl}$) are then separated by chromatography on silica gel and each isomer is deprotected with ammonia in methanol to give compounds of the present invention, *syn*adenol (1, $B = \text{adenin-}N^9\text{-yl}$) and *anti* isomer 2 ($B = \text{adenin-}N^9\text{-yl}$).

Syn- and *anti*-2-amino-6-chloro- N^9 -(2-hydroxymethylcyclopropylidenemethyl)purine 1 and 2 ($B = 2\text{-amino-6-chloropurin-}N^9\text{-yl}$) are separated by column chromatography on silica gel. The *syn* and *anti* isomers 1 and 2 ($B = 2\text{-amino-6-chloropurin-}N^9\text{-yl}$) are hydrolyzed with 80% formic acid (Harnden, M.R., et al., *J. Med. Chem.* 33:187-196 (1990)), to give compounds of the present invention, *synguanol* (1, $B = \text{guanin-}N^9\text{-yl}$) and *anti* isomer 2 ($B = \text{guanin-}N^9\text{-yl}$), as described in Figure 4.

Ammonolysis of the *syn*-2-amino-6-chloro- N^9 -(2-hydroxymethylcyclopropylidenemethyl)purine 1 ($B = 2\text{-amino-6-chloropurin-}N^9\text{-yl}$) affords *syn*-2,6-diamino- N^9 -(2-hydroxymethylcyclopropylidenemethyl)purine 1 ($B = 2,6\text{-diaminopurin-}N^9\text{-yl}$) as shown in Figure 4.

Reaction of *syn*-2-amino-6-chloro- N^9 -(2-hydroxymethylcyclopropylidenemethyl)purine 1 ($B = 2\text{-amino-6-chloropurin-}N^9\text{-yl}$) with cyclopropylamine gives *syn*-2-amino-6-cyclopropylamino- N^9 -(2-hydroxymethylcyclopropylidenemethyl)purine 1 ($B = 2\text{-amino-6-cyclopropylaminopurin-}N^9\text{-yl}$) as shown in Figure 4.

The mixture of *syn*- and *anti*-N¹-(2-hydroxymethylcyclopropylidenemethyl)-cytosines 1 and 2 (B = cytosin-N¹-yl) is benzoylated with a suitable benzoylating agent such as benzoic anhydride in an appropriate solvent such as ethanol (Otter, B.A., et al., N-Acyl Derivatives of 2'-Deoxycytidine in Synthetic Procedures in Nucleic Acid Chemistry, Vol. 1, John Wiley & Sons, New York, pp. 285-287 (1967)) to give *syn*- and *anti*-(N⁴-benzoyl)-N¹-(2-hydroxymethylcyclopropylidenemethyl)cytosines 1 and 2 (B = N⁴-benzoylcytosin-N¹-yl) which are separated by chromatography on silica gel as described in Figure 5. The separated isomers are debenzoylated with ammonia in methanol to afford *syn*- and *anti*-N¹-(2-hydroxymethylcyclopropylidenemethyl)cytosine 1 and 2 (B = cytosin-N¹-yl).

Prodrugs of *syn*- and *anti*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)-adenines 3 and 4 (B = adenin-N⁹-yl) were prepared by reaction of 1 and 2 (B = adenin-N⁹-yl) with phenylmethoxyalaninylphosphorochloridate (13) (McGuigan, C., et al., *Antiviral Res.* 17:311-321 (1992)) using a suitable catalyst, e.g., N-methylimidazole, in an appropriate organic solvent such as tetrahydrofuran and pyridine as described in Figures 6 and 7.

Compositions within the scope of invention include those comprising a novel compound of the present invention in an effective amount to achieve an intended purpose. Determination of an effective amount and intended purpose is within the skill of the art. Preferred dosages, which are dependent for example, on the severity of the infection and the individual patient's response to the treatment, can range from about 0.01 to about 100 mg/kg of body weight to give a blood concentration ranging from about 0.05 μ g to about 5 mg per mL of whole blood.

As used herein, the term "pharmaceutically acceptable salts" is intended to mean salts of the compounds of the present invention with pharmaceutically acceptable acids, e.g., inorganic acids such as sulfuric, hydrochloric, phosphoric, etc. or organic acids such as acetic.

Pharmaceutically acceptable compositions of the present invention may also include suitable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which may be used pharmaceutically. Such preparations, preferably those which can be administered orally, include tablets, dragees and capsules. Further preferred preparations are those which can be administered rectally, such as suppositories, as well as suitable solutions for administration by injection or orally, contain from about 0.1 to about

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99%, preferably about 25 to about 85%, of the active compound of the present invention, together with the excipient.

The pharmaceutical preparations of the present invention are manufactured in a manner which is itself known, *e.g.*, using the conventional mixing, granulating, dragee-making, dissolving or lyophilizing processes. Thus, pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipients, optionally grinding a resulting mixture and processing the mixture of granules, after adding suitable auxiliaries, if desired or necessary, to obtain tablets or dragee cores.

Suitable excipients are, in particular, fillers such as sugars, *e.g.*, lactose or sucrose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, *e.g.*, tricalcium diphosphate or calcium hydrogen phosphate, as well as binders such as starch paste, using, *e.g.*, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose and/or polyvinylpyrrolidone. If desired, disintegrating agents may be added such as the above-mentioned starches and also carboxymethyl starch, cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate. Auxiliaries are, above all, flow-regulating agents and lubricants, *e.g.*, silica, talc, stearic acid or salts thereof, such as magnesium stearate or calcium stearate, and/or polyethylene glycol. Dragee cores are provided with suitable coatings which, if desired, are resistant to gastric juices. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide, lacquer solutions and suitable organic solvent or solvent mixtures. In order to produce coatings resistant to gastric juices, solutions of suitable cellulose preparations, such as acetylcellulose phthalate or hydroxypropylmethylcellulose phthalate, are used. Dyestuffs or pigments may be added to the tablets or dragee coatings, *e.g.*, for identification or in order to characterize different combinations of active compound doses.

Other pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules may contain the active compounds in the form of granules which may be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and,

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optionally, stabilizers. In soft capsules, the active compounds are preferably dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be used.

Possible pharmaceutical preparations which can be used rectally include, e.g., suppositories, which consist of a combination of the active compounds with a suppository base. Suitable suppository bases are, e.g., natural or synthetic triglycerides, paraffin hydrocarbons, polyethylene glycols or higher alkanols. It is also possible to use gelatin rectal capsules which consist of a combination of the active compounds with a base. Possible base materials include, e.g., liquid triglycerides, polyethylene glycols or paraffin hydrocarbons.

Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, e.g., water-soluble salts. In addition, suspension of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, e.g., ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension such as sodium carboxymethylcellulose, sorbitol and/or dextran. Optionally, the suspension may also contain stabilizers.

Alternatively, the active compounds of the present invention may be administered in the form of liposomes, pharmaceutical compositions wherein the active compound is contained either dispersed or variously present in corpuscles consisting of aqueous concentrate layers adherent to hydrophobic lipidic layer. The active compound may be present both in the aqueous layer and in the lipidic layer or in the non-homogeneous system generally known as a lipophilic suspension.

It will be appreciated that the active compounds of the present invention may be administered in combination with known antiviral agents, e.g., acyclovir, ganciclovir, zidovudine, AZT, ddI, ddC and d4T.

The following Examples further describe the compounds of the present invention and the synthesis schemes for producing same.

EXAMPLE 1 - Synthesis of *cis* and *trans* Ethyl 2-Bromo-2-bromomethylcyclopropane-1-carboxylates (6 and 7).

Ethyl diazoacetate (90% in dichloromethane, 23.3 mL, 0.20 mol) was added into a solution of dirhodium tetraacetate (22.1 mg, 0.05 mmol) in 2,3-dibromopropene (5, 97%, 68.0 g, 0.34 mol) and CH₂Cl₂ (3 mL) with the aid of a syringe pump at a rate of 1.1 mL/hour with stirring at room temperature. The reaction

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mixture was distilled in vacuo and the distillate was trapped at -78°C to recover unreacted 2,3-dibromopropene (20 g, 29%). Water (100 mL) was added to the oily residue followed by a portionwise addition of powdered potassium permanganate at 0°C with stirring. A total of 30 g of permanganate was consumed. The excess of permanganate was removed by addition of solid sodium thiosulfate. The mixture was filtered using a Celite pad and the solids were washed with ether (4 x 80 mL) with the aid of a sonicator. The filtrate was extracted with ether (4 x 70 mL). The combined ether portions were washed with saturated aqueous sodium bicarbonate (2 x 100 mL), water (2 x 250 mL) and brine (2 x 100 mL). After drying with sodium sulfate and evaporation of ether a mixture of products 6 and 7 was obtained as a yellow oil (51.9 g, 91% yield), $n_D^{25} = 1.5131$. It was of sufficient purity to be used in the subsequent steps (Examples 2, 4, 6 and 10). The ^1H NMR spectrum indicated a mixture of *cis* and *trans* isomers 6 and 7 in the ratio of 1.5 : 1. Distillation of a sample of this product (5.0 g) gave colorless liquid (4.5 g), bp. 59-64°C/0.25 mmHg, $n_D^{25} = 1.5139$.

IR (neat): 1733 cm^{-1} (C=O, ester), 1036 and 868 cm^{-1} (cyclopropane ring).

^1H NMR (CDCl_3) δ , *cis* isomer 1.30 (t, 3, CH_3), 1.73 (t, 1) and 1.85 (dd, 1, H_3), 2.47 (dd, 1, H_2), 3.97 (d, 2, CH_2Br), 4.19 (q, 2, OCH_2); *trans* isomer 1.29 (t, 3, CH_3), 1.52 (dd, 1) and 1.89 (t, 1, H_3), 2.10 (dd, 1, H_2), 3.76 (d, 2, CH_2Br), 4.21 (q, 2, OCH_2).

^{13}C NMR (CDCl_3) ppm, *cis* isomer 14.14 (CH_3), 26.43 (C_3), 31.44 (C_1), 37.21 (C_2), 38.91 (CH_2Br), 61.66 (OCH_2), 169.32 (C=O); *trans* isomer 14.30 (CH_3), 22.34 (C_3), 29.01 (C_1), 35.86 (C_2), 42.02 (CH_2Br), 61.59 (OCH_2), 168.12 (C=O).

EI-MS 288 (M, 0.3), 286 (M, 1.9) and 284 (M, 0.5), 256 (1.6), 258 (2.6) and 260 (1.2), 243 (5.0), 241 (10.2) and 239 (5.3), 211 (7.0), 213 (13.8) and 215 (7.4), 205 (52.2) and 207 (51.0), 177 (96.7) and 179 (94.4), 159 (7.1) and 161 (7.0), 131 (15.2) and 133 (16.8), 97 (30.4), 81 (15.3), 69 (16.3), 53 (70.8), 39 (15.9), 28 (CO, 100.0);

HRMS: Calculated for $\text{C}_7\text{H}_{10}^{79}\text{Br}_2\text{O}_2$: M 283.90475. Found: M 283.9048.

Calculated for $\text{C}_7\text{H}_{10}\text{Br}_2\text{O}_2$: C, 29.59; H, 3.55; Br, 55.59. Found: C, 29.61; H, 3.62; Br, 55.37.

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EXAMPLE 2 - S y n t h e s i s o f c i s - N ⁹ - (1 - B r o m o - 2 - carbethoxycyclopropylmethyl)adenine and *trans*-N⁹-(1-Bromo-2-carbethoxycyclopropylmethyl)adenine 9 and 10.

A mixture of dibromoesters 6 and 7 (858 mg, 3.0 mmol) from Example 1 was
5 added into a suspension of sodium salt of adenine which was prepared from adenine (405 mg, 3.0 mmol) and sodium hydride (60% dispersion in mineral oil, 120 mg, 3.0 mmol) in N,N-dimethylformamide (25 mL) with stirring under nitrogen at room temperature. The resultant mixture was heated to 80°C (bath temperature) for 12 hours. The solvent was evaporated *in vacuo* (oil pump), the residue was adsorbed
10 on silica gel (3 g) which was then put on the top of a silica gel (70 g) column. The elution was performed with dichloromethane - methanol (96 : 4 and 94 : 6) the *cis* isomer 9 (305 mg, 29.9% yield) and *trans* isomer 10 (180 mg, 17.6% yield) were obtained as pale yellow solids. Analytical samples were obtained by recrystallization from benzene - ethyl acetate (*cis* isomer 9) and benzene (*trans* isomer 10).

15 **Cis isomer 9:**

Mp. 200 - 203°C.

UV (ethanol) max 260 nm (ϵ 14,900), 209 nm (ϵ 19,500).

IR (KBr) 3340 and 3150 cm^{-1} (NH_2), 1725 cm^{-1} (C=O, ester), 1670, 1600 and 1580 cm^{-1} (adenine ring), 1045, 1015 and 867 cm^{-1} (cyclopropane ring).

20 ^1H NMR (CD_3SOCD_3) δ 1.23 (t, 3, CH_3), 1.76 (dd, 1) and 2.00 (t, 1, H_4), 2.49 (dd, 1, H_3 of cyclopropane), 4.18 (q, 2, OCH_2), 4.66 (s, 2, H_1), 7.24 (s, 2, NH_2), 8.10 (s, 2, H_2 and H_8 of adenine).

^{13}C NMR (CD_3SOCD_3) ppm 13.52 (CH_3), 22.25 (C_4), 28.57 ($\text{C}3'$), 36.28 (C_2), 46.64 (C_1), 60.75 (OCH_2), 168.82 (C=O); adenine ring: 118.10 (C_5), 140.46 (C_8),
25 149.20 (C_4), 152.03 (C_2), 155.49 (C_6).

FAB-MS (thioglycerol matrix) 341 and 343 ($\text{M}+2\text{H}$, 20.3, 18.6), 340 and 342 ($\text{M} + \text{H}$, 98.4, 100.0), 262 (8.0), 214 (4.3), 188 (5.9), 136 (25.2).

Calculated for $\text{C}_{12}\text{H}_{14}\text{BrN}_5\text{O}_2$: C, 42.47; H, 4.16; Br, 23.28; N, 20.65. Found: C, 42.24; H, 4.29; Br, 23.15; N, 20.59.

30 ***Trans* isomer 10:**

Mp. 186 - 189°C.

UV (ethanol) max 260 nm (ϵ 14,700), 209 nm (ϵ 18,700).

IR (KBr) 3350 and 3160 cm^{-1} (NH_2), 1745 cm^{-1} (C=O, ester), 1655, 1605 and 1585 cm^{-1} (adenine ring), 1045, 1015 and 865 cm^{-1} (cyclopropane ring).

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^1H NMR (CDCl_3) δ 1.14 (t, 3, CH_3), 1.57 (t, 1) and 1.93 (dd, 1, H_4), 2.64 (dd, 1, H_3), 4.07 (q, 2, OCH_2), 4.52 (d, 2, H_1), 7.27 (s, 2, NH_2), 8.13 and 8.17 (2s, 2, H_2 and H_8 of adenine).

^{13}C NMR (CDCl_3) ppm 13.70 (CH_3), 19.95 (C_4), 26.05 (C_3), 36.99 (C_2), 51.93 (C_1), 60.42 (OCH_2), 167.63 (C=O); adenine ring: 118.01 (C_5), 140.30 (C_8), 149.23 (C_4), 152.14 (C_2), 155.55 (C_6).

FAB-MS (thioglycerol matrix) 341 and 343 ($\text{M} + 2\text{H}$, 19.0, 18.2), 340 and 342 ($\text{M} + \text{H}$, 96.3, 100.0), 262 (32.1), 214 (12.1), 188 (12.5), 136 (78.3).

Calculated for $\text{C}_{12}\text{H}_{14}\text{BrN}_5\text{O}_2$: C, 42.47; H, 4.16; Br, 23.28; N, 20.65. Found:
10 C, 42.60; H, 4.34; Br, 23.14; N, 20.87.

EXAMPLE 3 - Synthesis of *syn*-N⁹-(2-Carbethoxycyclopropylidenemethyl)adenine (11, B = adenine-N⁹-yl, R = ethyl) and *anti*-N⁹-(2-Carbethoxycyclopropylidenemethyl)adenine (12, B = adenine-N⁹-yl, R = ethyl) Using Bromoesters 9 and 10 as Starting Materials.
15

Solid potassium tert.-butoxide (505 mg, 4.5 mmol) was added into a solution of bromoesters 9 and 10 from Example 2 (1.02 g, 1.023 mmol) in DMF (35 mL) at 0°C with stirring under nitrogen. The stirring continued for 2 hours. The reaction mixture was then added dropwise into saturated aqueous ammonium chloride (20 mL) at 0°C with stirring. After 15 minutes, the solvents were removed by evaporation *in vacuo* at room temperature. The residue was chromatographed on a silica gel column using dichloromethane - methanol (9 : 1) as an eluent to give the mixture of compounds 11 and 12 (B = adenine-N⁹-yl, R = ethyl, 545 mg, 70% yield) as a white powder. Mp. 166 - 175°C after recrystallization from benzene. The ^1H NMR indicated
20 the presence of *syn* and *anti* isomers 10 and 11 (B = adenine-N⁹-yl, R = ethyl) in the ratio of 1.5 : 1.
25

UV (ethanol) max 276 nm (shoulder, ϵ 7,600), 256 nm (shoulder, ϵ 11,800), 228 nm (ϵ 22,500).

IR (KBr) 3350 and 3160 cm^{-1} (NH_2), 1735 cm^{-1} (C=O , ester), 1660, 1605 and
30 1585 cm^{-1} (adenine ring and olefin).

^1H NMR (CD_3SOCD_3) δ , *syn* isomer 11 (B = adenine-N⁹-yl, R = ethyl) 1.13 (t, 3, CH_3), 1.91 (ddd, 1) and 2.00 (t d, 1, H_4), 2.89 (ddd, 1, H_3), 4.02 - 4.13 (m, 2, OCH_2), 7.40 (s, 2, NH_2), 7.54 (q, 1, H_1), 8.15 (s, 1) and 8.23 (s, 1, H_2 and H_8 of adenine); *anti* isomer 12 (B = adenine-N⁹-yl, R = ethyl) 1.19 (t, 3, CH_3), 2.07 (ddd, 1) and 2.17 (t d, 1, H_4), 2.64 (ddd, 1, H_3), 4.02 - 4.13 (m, 2, OCH_2), 7.39 (s, 2, NH_2),
35 7.59 (q, 1H, H_1), 8.18 (s, 1, H_2 of adenine), 8.52 (s, 1, H_8 of adenine).

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¹³C NMR (CD₃SOCD₃) ppm, *syn* isomer 11 (B = adenine-N⁹-yl, R = ethyl) 10.70 (C₄), 14.52 (CH₃), 19.78 (C-3'), 61.10 (OCH₂), 111.71 (C₁), 112.85 (C₂), 170.71 (C=O); adenine ring: 118.90 (C₅), 137.75 (C₈), 148.76 (C₄), 153.64 (C₂), 156.56 (C₆); *anti* isomer 12 (B = adenine-N⁹-yl, R = ethyl) 13.30 (C₄), 14.52 (CH₃), 17.52 (C₃), 61.10 (OCH₂), 112.18 (C₁), 113.16 (C₂), 171.29 (C=O); adenine ring: 118.90 (C₅), 137.99 (C₈), 148.76 (C₄), 153.64 (C₂), 156.56 (C₆).

EI-MS 259 (M, 54.2), 245 (6.1), 230 (24.3), 214 (28.4), 202 (M, 9.9), 187 (100.0), 160 (19.6), 135 (22.4).

Calculated for C₁₂H₁₃N₅O₂: C, 55.58; H, 5.06; N, 27.02%. Found: C, 55.60; H, 4.82; N, 27.28%.

EXAMPLE 4 - Direct Synthesis of *syn*-N⁹-(2-Carbethoxycyclopropylidenemethyl)adenine (11, B = adenin-N⁹-yl, R = ethyl) and *anti*-N⁹-(2-Carbethoxycyclopropylidenemethyl)adenine (12, B = adenin-N⁹-yl, R = ethyl) from Adenine.

A mixture of adenine (810 mg, 6.0 mmol), *cis*- and *trans* ethyl 2-bromo-2-bromomethylcyclopropane 1-carboxylates (6 and 7, 2.14 g, 7.5 mmol) from Example 1 and flame-dried potassium carbonate (4.98 g, 36.0 mmol) in N,N-dimethylformamide (30 mL) was stirred at 100 °C under nitrogen for 22 hours. After cooling, the insoluble portion was filtered off and it was washed with a mixture of dichloromethane - ethanol (4 : 1, 2 x 50 mL). The filtrate was evaporated and the residue was flash-chromatographed on a silica gel column with dichloromethane - methanol (95 : 5) as an eluent. Evaporation of appropriate fractions gave a mixture of products 11 and 12 (B = adenin-N⁹-yl, R = ethyl) as a pale yellow solid (610 mg, 39.2% yield), mp. 166 - 175°C in the ratio of 2 : 1 as determined by ¹H NMR.

EXAMPLE 5 - Synthesis of Synadenol (1, B = adenin-N⁹-yl) and *anti*-N⁹-(2-Hydroxymethylcyclopropylidenemethyl)adenine (2, B = adenin-N⁹-yl).

Diisobutylaluminum hydride in tetrahydrofuran (1 M, 27.2 mL, 27.2 mmol) was added dropwise into a solution of esters 11 and 12 (B = adenin-N⁹-yl, R = ethyl, 881 mg, 3.4 mmol) from Example 3 or 4 in tetrahydrofuran (50 mL) at 0°C over 10 minutes with stirring under nitrogen. The stirring at 0°C was continued for 3.5 hours. Saturated aqueous ammonium chloride (25 mL) was then added with stirring at 0°C. The gel-like solid was filtered off using a Celite bed and it was washed with dichloromethane - methanol (1 : 1, 5 x 40 mL). The combined filtrate and washings were evaporated and the residue was chromatographed on a silica gel column using dichloromethane - methanol (9 : 1 and 85 : 15) as eluents. The appropriate fractions

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were evaporated to give a mixture of compounds 1 and 2 (B = adenin-N⁹-yl) as a pale yellow solid (557 mg, 75% yield).

A solution of this mixture of products (557 mg, 2.56 mmol) in N,N-dimethylformamide (30 mL) and N,N-dimethylformamide dimethyl acetal (0.51 mL, 3.85 mmol) was stirred at room temperature for 14 hours. Evaporation of the volatile components *in vacuo* at room temperature (oil pump) left a mixture of compound 1 and 2 (B = N⁶-dimethylaminomethyleneadenin-N⁹-yl) as a viscous yellow sirup which was flash-chromatographed on a silica gel column using dichloromethane - methanol (92 : 8 and 85 : 15). The appropriate fractions were pooled and evaporated to give *syn* isomer 1 (B = N⁶-dimethylaminomethyleneadenin-N⁹-yl), 264 mg, 37.8% yield) as a white solid and *anti* isomer 2 (B = N⁶-dimethylaminomethyleneadenin-N⁹-yl, 260 mg, 37.2% yield) as a foam. A small portion of 1 and 2 (B = N⁶-dimethylaminomethyleneadenin-N⁹-yl, 63 mg, 9.0% yield) was recovered as a mixture.

15 *Syn* isomer 1 (B = N⁶-dimethylaminomethyleneadenin-N⁹-yl):

Mp. 172 - 178°C.

UV (ethanol) max 311nm (ϵ 31,300), 233 nm (ϵ 27,600).

IR (KBr) 3500 - 3180 cm⁻¹ (OH), 1638 and 1600 - 1540 cm⁻¹ (olefin and N⁶-dimethylaminomethyleneadenine), 1060, 1030, 830 and 810 cm⁻¹ (cyclopropane ring).

¹H NMR (CD₃SOCD₃) δ 1.21 (t, 1) and 1.49 (t, 1, H₄), 2.14 (dq, 1, H₃), 3.17 and 3.10 /2s, 6, N(CH₃)₂/, 3.29 (t, overlapped with H₂O, 1) and 3.74 (dt, 1, H₅), 5.11 (t, 1, OH), 7.43 (s, 1, H₁), 8.42 (s, 1, H₂ of purine), 8.86 (s, 1, H₈ of purine), 8.90 /s, 1, N=CHN(CH₃)₂/.

25 ¹³C NMR (CD₃SOCD₃) ppm 6.74 (C₄), 19.73 (C₃), 35.00 and 41.13 /N(CH₃)₂/, 63.38 (C₅), 110.60 (C₁), 116.18 (C₂), 152.77 /N=CHN(CH₃)₂/; purine ring: 125.38 (C₅), 139.98 (C₈), 150.40 (C₄), 158.53 (C₂), 159.66 (C₆).

FAB-MS (thioglycerol matrix) 381 (M + thioglycerol + H, 5.7), 273 (M + H, 7.4), 191 (8.2), 149 (29.8), 91 (100.0).

30 *Anti* isomer 2 (B = N⁶-dimethylaminomethyleneadenin-N⁹-yl):

UV (ethanol) max 310 nm (ϵ 32,300), 231 nm (ϵ 29,500).

IR (KBr) 3550 - 3200 cm⁻¹(OH), 1635 and 1600 - 1540 cm⁻¹(olefin and N⁶-dimethylaminomethyleneadenine), 1030 and 810 cm⁻¹ (cyclopropane ring).

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^1H NMR (CD_3SOCD_3) δ 1.39 (ddd, 1) and 1.71 (td, 1, H_4), 1.97 (dq, 1, H_3), 3.17 and 3.11 /2s, 6, $\text{N}(\text{CH}_3)_2$ /, 3.41 (t, 2, H_5), 4.82 (t, 1, OH), 7.52 (d, 1, H_1), 8.43 (s, 1H, H_2 of purine), 8.56 (s, 1, H_8 of purine), 8.89 /s, 1, $\text{N}=\text{CHN}(\text{CH}_3)_2$ /.

^{13}C NMR (CD_3SOCD_3) ppm 7.93 (H_4), 18.09 (H_3), 35.04 and 41.14 / $\text{N}(\text{CH}_3)_2$ /, 63.57 (C_5), 110.74 (C_1), 116.59 (C_2), 152.80 / $\text{N}=\text{CHN}(\text{CH}_3)_2$ /, purine ring: 125.40 (C_5), 139.47 (C_8), 150.59 (C_4), 158.48 (C_2), 159.67 (C_6).

FAB-MS (thioglycerol matrix) 381 ($\text{M} + \text{thioglycerol} + \text{H}$, 51.7), 273 ($\text{M} + \text{H}$, 100.0), 191 (69.8).

The separated isomers (250 mg, 0.92 mmol of each) were dissolved in methanolic ammonia saturated at 0°C (100 mL). The mixtures were stirred at room temperature for 16 hours. The volatile components were evaporated and the residues were recrystallized from methanol to give synadenol (1, B = adenin- N^9 -yl, 170 mg, 85% yield) and the *anti* isomer 2 (B = adenin- N^9 -yl, 172 mg, 86% yield). Synadenol 1 (B = adenin- N^9 -yl):

15 Mp. $238 - 239^\circ\text{C}$ (change of modification at $200 - 231^\circ\text{C}$).

UV (ethanol) max 276 nm (shoulder, ϵ 9,300), 261 nm (ϵ 13,500), 226 nm (ϵ 29,400).

IR (KBr): 3300 and 3100 cm^{-1} (NH_2 and OH), 1670, 1600 and 1580 cm^{-1} (adenine ring and olefin), 1047 and 1025 cm^{-1} (cyclopropane ring).

20 ^1H NMR (CD_3SOCD_3) δ 1.22 (m, 1) and 1.50 (dt, 1, H_4), 2.48 (m, 1, H_3), 3.33 (m, overlapped with H_2O) and 3.73 (qt, 1, H_5), 5.11 (t, 1, OH), 7.33 (s, 2, NH_2), 7.48 (d, 1, H_1), 8.17 (s, 1, H_2 of adenine), 8.74 (s, 1, H_8 of adenine).

^{13}C NMR (CD_3SOCD_3) ppm 6.72 (C_4), 19.70 (C_3), 63.35 (C_5), 110.69 (C_1), 115.87 (C_2); adenine ring: 118.84 (C_5), 138.13 (C_8), 148.13 (C_4), 153.42 (C_2), 156.47 (C_6).

EI-MS 217 (M , 31.4), 200 (45.2), 187 (37.0), 173 (6.5), 159 (11.2), 148 (15.3), 135 (100.0).

HRMS: Calculated for $\text{C}_{10}\text{H}_{11}\text{N}_5\text{O}$: M , 217.0964. Found: M , 217.0960.

30 Calculated for $\text{C}_{10}\text{H}_{11}\text{N}_5\text{O}$: C, 55.29; H, 5.10; N, 32.24. Found: 55.32; H, 4.99; N, 32.44.

Anti isomer 2 (B = adenin- N^9 -yl):

Mp. $208 - 210^\circ\text{C}$.

UV (ethanol) max 277 nm (shoulder, ϵ 8,900), 261 nm (ϵ 12,200), 226 nm (ϵ 27,600).

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IR (KBr) 3340 and 3180 cm^{-1} (NH_2 and OH), 1666, 1612, and 1578 cm^{-1} (adenine ring and olefin).

^1H NMR (CD_3SOCD_3) δ 1.38 (m, 1) and 1.71 (dt, 1, H_4), 1.97 (dqt, 1, H_3), 3.41 (t, 2, H_5), 4.82 (t, 1, OH), 7.34 (s, 2, NH_2), 7.48 (q, 1, H_1), 8.17 (s, 1, H_2 of adenine), 8.48 (s, 1, H_8 of adenine).

^{13}C NMR (CD_3SOCD_3) ppm 9.68 (C_4), 18.06 (C_3), 63.59 (C_5), 110.81 (C_1), 116.24 (C_2); adenine ring: 118.85 (C_5), 137.61 (C_8), 148.68 (C_4), 153.48 (C_2), 156.49 (C_6).

EI-MS 217 (M, 15.0), 200 (78.5), 172 (25.9), 148 (11.3), 136 (43.1), 41 (100.0).

HRMS: Calculated for $\text{C}_{10}\text{H}_{11}\text{N}_5\text{O}$: M 217.0964; Found: M 217.0954.

Calculated for $\text{C}_{10}\text{H}_{11}\text{N}_5\text{O}$: C, 55.29; H, 5.10; N, 32.24. Found: C, 55.41; H, 5.20; N, 32.26.

EXAMPLE 6 - Synthesis of *syn*-2-Amino-6-chloro- N^9 -(2-carbethoxycyclopropylidenemethyl)purine (11, B = 2-amino-6-chloropurin- N^9 -yl, R = ethyl) and *anti*-2-Amino-6-chloro- N^9 -(2-carbethoxycyclopropylidenemethyl)purine (12, B = 2-amino-6-chloropurin- N^9 -yl, R = ethyl) from 2-Amino-6-chloropurine.

A mixture of 2-amino-6-chloropurine (8, B = 2-amino-6-chloropurin- N^9 -yl, 2.04 g, 12 mmol), dibromoesters 6 + 7 (5.29 g, 15 mmol) from Example 1 and flame-dried potassium carbonate (8.29 g, 60 mmol) in N,N-dimethylformamide (60 mL) was stirred at 100°C (bath temperature) for 20 hours under nitrogen. Chromatography on a silica gel column (80 g) using dichloromethane - methanol (98.5 : 1.5 and 97 : 3) gave a crude mixture of esters 11 and 12 (B = 2-amino-6-chloropurin- N^9 -yl, R = ethyl, 1.98 g, 56.2% yield) in the ratio of 2 : 1 as indicated by ^1H NMR as a yellow solid.

Mp. 177 - 196°C after recrystallization from benzene or 171 - 187°C after recrystallization from ethanol.

UV (ethanol) max 311 nm (ϵ 7,400), 230 nm (ϵ 26,800), 210 nm (shoulder, ϵ 16,100).

IR (KBr) 3420, 3325 and 3220 cm^{-1} (NH_2), 1725 cm^{-1} (C=O, ester), 1646, 1615 and 1565 cm^{-1} (purine ring and olefin), 1000 cm^{-1} (cyclopropane ring).

^1H NMR (CD_3SOCD_3) δ , *syn* isomer 11 (B = 2-amino-6-chloropurin- N^9 -yl, R = ethyl) 1.10 (t, 3, CH_3), 1.91 (ddd, 1) and 2.00 (td, 1, H_4), 2.90 (ddd, 1, H_3), 4.02 - 4.12 (m, 2, OCH_2), 6.99 (s, 2, NH_2), 7.35 (q, 1, H_1), 8.19 (s, 1, H_8 of purine); *anti* isomer 12 (B = 2-amino-6-chloropurin- N^9 -yl, R = ethyl) 1.18 (t, 3, CH_3), 2.07 (ddd,

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1) and 2.17 (ddd, 1, H₄), 2.63 (ddd, 1, H₃), 4.02 - 4.12 (m, 2, OCH₂), 7.04 (s, 2, NH₂), 7.42 (q, 1, H₁), 8.46 (s, 1, H₈ of purine).

¹³C NMR (CD₃SOCD₃) ppm, *syn* isomer 11 (B = 2-amino-6-chloropurin-N⁹-yl, R = ethyl) 10.75 (C₄), 14.42 (CH₃), 19.76 (C₃), 61.17 (OCH₂), 111.17 (C₁), 113.74 (C₂), 170.58 (C=O); purine ring: 123.49 (C₅), 139.75 (C₈), 150.27 (C₄), 152.95 (C₂), 160.55 (C₆); *anti* isomer 12 (B = 2-amino-6-chloropurin-N⁹-yl, R = ethyl) 13.45 (C₄), 14.50 (CH₃), 17.67 (C₃), 61.09 (OCH₂), 111.84 (C₁), 114.11 (C₂), 170.14 (C=O); purine ring: 123.57 (C₅), 140.23 (C₈), 150.19 (C₄), 153.10 (C₂), 160.55 (C₆).

EI-MS 293 and 295 (M 89.4, 29.9), 264 and 266 (30.8, 11.5), 248 and 250 (22.7, 8.7), 236 and 238 (10.9, 4.0), 221 and 223 (100.0, 32.9), 208 and 210 (5.9, 3.2), 184 (25.3), 169 and 171 (39.1, 14.1).

HRMS Calculated for C₁₂H₁₂³⁵ClN₅O₂: M 293.06795. Found: M 293.0680.

Calculated for C₁₂H₁₂ClN₅O₂: C, 49.14; H, 4.13; Cl, 11.93; N, 23.89. Found: C, 49.00; H, 4.27; Cl, 12.11; N, 24.18.

EXAMPLE 7 - Synthesis of *syn*-2-Amino-6-chloro-N⁹-(2-carbethoxycyclopropylidenemethyl)purine (1, B = 2-amino-6-chloropurin-N⁹-yl) and *anti*-2-Amino-6-chloro-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine (1, B = 2-amino-6-chloropurin-N⁹-yl).

Diisobutylaluminum hydride in cyclohexane (1 M, 19.4 mL, 19.4 mmol) was added dropwise into a solution of esters 11 and 12 (B = 2-amino-6-chloropurin-N⁹-yl, R = ethyl, 1.90 g, 6.47 mmol) from Example 6 in tetrahydrofuran (100 mL) during 10 min. with stirring at 0°C under nitrogen. Two additional portions of hydride (6.47 mL, 6.47 mmol) were added in 30 min. intervals. The stirring at 0°C was continued for a total of 2 hours. The reaction was carefully quenched by adding aqueous methanol (50%, 10 mL) with stirring which was continued for 30 min. at 0°C and then overnight at room temperature. The precipitated gel was filtered off and it was washed thoroughly with dichloromethane - methanol (4 : 1, 2 × 50 mL). The combined filtrate and washings were evaporated and the residue was chromatographed on a silica gel column using dichloromethane - methanol (97 : 3 and 92 : 8). The fractions of separated products 1 and 2 (B = 2-amino-6-chloropurin-N⁹-yl) were rechromatographed to give *syn* isomer 1 (B = 2-amino-6-chloropurin-N⁹-yl, 680 mg, 41.8% yield) and *anti* isomer 2 (B = 2-amino-6-chloropurin-N⁹-yl, 380 mg, 23.3% yield). Analytical samples of both isomers were obtained by recrystallization from ethanol.

Syn isomer 1 (B = 2-amino-6-chloropurin-N⁹-yl):

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Mp. 214 - 216°C.

UV (ethanol) max 310 nm (ϵ 8,000), 233 nm (ϵ 31,800).

IR (KBr) 3440 (shoulder), 3330 and 3200 cm^{-1} (OH and NH_2), 1640, 1610 and 1570 cm^{-1} (purine ring and olefin), 1040 and 1005 cm^{-1} (cyclopropane ring).

5 ^1H NMR (CD_3SOCD_3) δ 1.21 (ddd, 1) and 1.49 (td, 1, H_4), 2.13 (dq, 1, H_3), 3.28 (ddd, overlapped with H_2O at 3.30, 1) and 3.73 (dt, 1, H_5), 5.06 (dd, 1, OH), 7.01 (s, 2, NH_2), 7.20 (q, 1, H_1), 8.70 (s, 1, H_8 of purine).

^{13}C NMR (CD_3SOCD_3) ppm 6.80 (C_4), 19.73 (C_3), 63.25 (C_5), 110.24 (C_1), 116.81 (C_2); purine ring: 123.52 (C_5), 140.36 (C_8), 150.00 (C_4), 152.81 (C_2), 160.49
10 (C_6).

EI-MS 251 and 253 (M, 41.4, 15.1), 234 and 236 (50.9, 19.8), 221 and 223 (18.6, 8.9), 198 (26.2), 170 and 172 (100.0, 38.5).

Calculated for $\text{C}_{10}\text{H}_{10}\text{ClN}_5\text{O}$: C, 47.80; H, 4.01; Cl, 13.93; N, 27.89. Found: C, 47.70; H, 4.07; Cl, 14.18; N, 27.86.

15 *Anti* isomer 2 (B = 2-amino-6-chloropurin- N^9 -yl):

Mp 201 - 204°C.

UV (ethanol) max 310 nm (ϵ 8,100), 231 nm (ϵ 33,700).

IR (KBr) 3460, 3340 and 3220 cm^{-1} (OH and NH_2), 1650-1610 and 1568 cm^{-1} (purine ring and olefin), 1010 cm^{-1} (cyclopropane ring).

20 ^1H NMR (CD_3SOCD_3) δ 1.37 (ddd, 1) and 1.71 (td, 1, H_4), 1.96 (dq, 1, H_3), 3.40 (d of AB system, 2, H_5), 4.82 (t, 1, OH), 7.01 (s, 2, NH_2), 7.31 (q, 1, H_1), 8.48 (s, 1, H_8 of purine).

^{13}C NMR (CD_3SOCD_3) ppm 9.79 (H_4), 18.16 (C_3), 63.52 (C_5), 110.44 (C_1), 117.37 (C_2); purine ring: 123.51 (C_5), 140.00 (C_8), 150.05 (C_4), 152.99 (C_2), 160.48
25 (C_6).

EI-MS 251 and 253 (M, 36.1, 12.3), 234 and 236 (84.0, 29.7), 222 and 224 (8.6, 3.4), 198 (24.6), 170 and 172 (100.0, 37.8).

Calculated for $\text{C}_{10}\text{H}_{10}\text{ClN}_5\text{O}$: C, 47.80; H, 4.01; Cl, 13.93; N, 27.89. Found: C, 47.88; H, 4.10; Cl, 14.02; N, 27.91.

30 **EXAMPLE 8 - Synthesis of Synguanol (1, B = guanin- N^9 -yl) and *anti*- N^9 -(2-Hydroxymethylcyclopropylidenemethyl)guanine (2, B = guanin- N^9 -yl).**

The solution of the *syn* isomer 1 (B = 2-amino-6-chloropurin- N^9 -yl, 179 mg, 0.78 mmol) from Example 7 in aqueous formic acid (80%, 10 mL) was heated at
35 80°C (bath temperature) with stirring for 3.5 hours. After cooling, the volatile

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components were evaporated, the residue was dissolved in water (20 mL) and the solution was lyophilized. The resultant white solid was suspended in methanol - concentrated aqueous ammonia (3 : 1, 40 mL) and the mixture was stirred overnight at room temperature. The solvents were evaporated, and suspension of the residue in methanol (70 mL) was refluxed for 2 hours. The mixture was kept overnight at 0°C to give synguanol (1, B = guanine-N⁹-yl, 155 mg, 85% yield).

Mp. >300°C.

UV (ethanol) max 271 nm (ϵ 11,600), 229 nm (ϵ 29,400).

IR (KBr) 3450 (flat peak), 3320 and 3170 cm⁻¹ (OH and NH₂), 1725, 1690, 1640 and 1600 cm⁻¹ (guanine ring and olefin), 1020 cm⁻¹ (cyclopropane ring).

¹H NMR (CD₃SOCD₃) δ 1.17 (ddd, 1) and 1.45 (td, 1, H₄), 2.07 (dq, 1, H₃ of cyclopropane), 3.32 (t, overlapped with H₂O at 3.30, 1) and 3.68 (dd, 1, H₅), 5.04 (broad s, 1, OH), 6.51 (s, 2, NH₂), 7.11 (d, 1, H₁), 8.31 (s, 1, H₈ of purine), 10.65 (s, 1, NH).

¹³C NMR (CD₃SOCD₃) ppm 6.60 (C₄), 19.56 (C₃), 63.23 (C₅), 110.59 (C₁), 115.44 (C₂); guanine ring: 116.68 (C₅), 134.70 (C₈), 150.12 (C₄), 154.33 (C₂), 157.11 (C₆).

FAB-MS (thioglycerol matrix) 342 (M + thioglycerol + H, 10.4), 234 (M + H, 3.9), 233 (M, 0.9), 232 (M - H, 3.9), 216 (6.6), 197 (15.3), 181 (52.0).

Calculated for C₁₀H₁₁N₅O₂: C, 51.48; H, 4.76; N, 30.04. Found: C, 51.60; H, 4.90; N, 29.89.

The procedure described above was used for the synthesis of the *anti* isomer 2 (B = guanine-N⁹-yl, 120 mg, 83% yield) starting from the chloropurine intermediate 10 (B = 2-amino-6-chloropurin-N⁹-yl, 150 mg, 0.60 mmol) from Example 7.

Mp. >300°C.

UV (ethanol) max 270 nm (ϵ 11,900), 228 nm (ϵ 30,300).

IR (KBr) 3440 - 3300 and 3150 cm⁻¹ (OH and NH₂), 1710 - 1640, and 1610 cm⁻¹ (guanine ring and olefin), 1030 cm⁻¹ (cyclopropane ring).

¹H NMR (CD₃SOCD₃) δ 1.31 (ddd, 1) and 1.66 (td, 1, H₄), 1.92 (dq, 1, H₃), 3.37 (dd, 2, H₅), 4.80 (broad s, 1, OH), 6.52 (s, 2, NH₂), 7.21 (q, 1, H₁), 8.04 (s, 1, H₈ of purine) and 10.66 (s, 1, NH).

¹³C NMR (CD₃SOCD₃) ppm 9.55 (C₄), 17.91 (C₃), 63.58 (C₅), 110.75 (C₁), 115.89 (C₂); guanine ring: 116.68 (C₅), 134.16 (C₈), 150.29 (C₄), 154.35 (C₂) and 157.10 (C₆).

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FAB-MS (thioglycerol matrix) 342 (M + thioglycerol + H, 44.5), 234 (M + H, 35.5), 216 (9.6), 197 (10.3), 181 (41.8), 152 (46.0), 91 (64.8), 73 (100.0).

Calculated for $C_{10}H_{11}N_5O_2 \cdot 0.5 H_2O$: C, 49.58; H, 4.99; N, 28.91. Found: C, 49.34; H, 5.09; N, 28.70.

5 **EXAMPLE 9 - Synthesis of *syn*-2,6-Diamino-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine (1, B = 2,6-diaminopurin-N⁹-yl).**

A mixture of compound 1 (B = 2-amino-6-chloropurin-N⁹-yl, 140 mg, 0.56 mmol) from Example 7 and ammonia in methanol (saturated at 0°C, 80 mL) was
10 heated in a stainless steel bomb at 95°C (bath temperature) for 16 hours. After cooling, the volatile components were evaporated and the residue was chromatographed on silica gel with dichloromethane - methanol (4 : 1) to give the title compound 1 (B = 2,6-diaminopurin-N⁹-yl, 105 mg, 81.4% yield) as a white solid.

Mp. 218 - 221°C.

15 UV (ethanol) max 280 nm (ϵ 9,300), 220 nm (ϵ 27,900).

IR (KBr) 3495, 3430, 3360 and 3160 cm^{-1} (NH₂ and OH), 1660, 1630 and 1600 cm^{-1} (purine ring), 1035 and 793 cm^{-1} (cyclopropane ring).

¹H NMR (CD₃SOCD₃) δ 1.16 (ddd, 1) and 1.44 (td, 1, H₄), 2.10 (dqd, 1, H₃), 3.33 (dt, overlapped with H₂O at 3.30, 1) and 3.67 (dt, 1, H₅), 5.07 (t, 1, OH), 5.87
20 (s, 2, 2-NH₂), 6.76 (s, 2, 6-NH₂), 7.16 (d, 1, H₇), 8.31 (s, 1, H₈ of purine).

¹³C NMR (CD₃SOCD₃) ppm 6.65 (C_{4'}), 19.62 (C_{3'}), 63.43 (C_{5'}), 110.83 (C_{1'}), 113.22 (C_{2'}); purine ring: 113.86 (C₅), 134.67 (C₈), 150.82 (C₄), 156.58 (C₂), 161.01 (C₆).

EI-MS 232 (M, 45.2), 215 (39.7), 202 (18.5), 198 (10.8), 173 (7.9), 163 (10.9),
25 159 (9.5), 150 (100.0).

HRMS Calculated for $C_{10}H_{12}N_6O$: M 232.1072. Found: M 232.1076.

Calculated for $C_{10}H_{12}N_6O$: C, 51.70; H, 5.21; N, 36.20. Found: C, 51.53; H, 5.35; N, 36.35.

30 **EXAMPLE 10 - Synthesis of *syn*-2-Amino-6-cyclopropylamino-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine (1, B = 2-amino-6-cyclopropylaminopurin-N⁹-yl).**

A solution of compound 1 (B = 2-amino-6-chloropurin-N⁹-yl, 151 mg, 0.60 mmol) and cyclopropylamine (0.416 mL, 6.0 mmol) in ethanol (15 mL) was refluxed for 24 hours. After cooling, the volatile components were evaporated and the
35 residue was chromatographed on silica gel with dichloromethane - methanol (95: 5

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→ 9:1) to give the title compound 1 (B = 2-amino-6-cyclopropylaminopurin-N⁹-yl, 156 mg, 95.5% yield) as a white solid.

Mp. 190-193° C.

UV (ethanol) max 285 nm (ϵ 16,000), 224 nm (ϵ 40,000).

5 IR (KBr) 3475, 3330 and 3200 cm⁻¹ (NH₂ and OH) and 1640-1595 cm⁻¹ (olefin and purine ring), 1043 cm⁻¹ (cyclopropane ring).

¹H NMR (CD₃SOCD₃) δ 0.52-0.59 (m, 2) and 0.63 (d t, 2, CH₂ of cyclopropylamino group), 1.14 (ddd, 1) and 1.43 (t d, 1, H₄), 2.06 (dq, 1, H₃), 3.00 (broad s, 1, CH of cyclopropylamino group), 3.32 (m, overlapped with H₂O at 3.32, 1) and 3.66 (qt, 1, H₅), 5.05 (t, 1, OH), 5.94 (s, 2, 2-NH₂), 7.16 (d, 1, H₁), 7.38 (d, 1, 6-NH), 8.30 (s, 1, H₈).

¹³C NMR (CD₃SOCD₃) ppm 6.60 (C₄), 6.84 (CH₂ of the cyclopropylamino group), 19.57 (C₃), 63.41 (C₅), 110.79 (C₁), 113.44 (C₂), 113.88 (C₅), 134.44 (C₈), 150.31 (C₄), 156.33 (C₂) and 160.91 (C₆).

15 EI-MS 272 (M, 93.8), 257 (34.9), 255 (29.5), 242 (13.2), 227 (23.8), 213 (15.5), 189 (33.1), 175 (59.5), 162 (25.1), 134 (30.1), 43 (100.0).

HRMS Calculated for C₁₃H₁₆N₆O: M 272.1386. Found: 272.1384.

Calculated for C₁₃H₁₆N₆O: C, 57.32; H, 5.93; N, 30.87. Found: C, 57.10; H, 6.07; N, 30.63.

20 **EXAMPLE 11 - Synthesis of syn - N¹ - (2 - Carbomethoxycyclopropylidenemethyl)cytosine (11, B = cytosin-N¹-yl, R = methyl) and anti-N¹-(2-Carbomethoxycyclopropylidenemethyl)cytosine (12, B = cytosin-N¹-yl, R = methyl).**

25 A mixture of N⁴-acetylcytosine (8, B = N⁴-acetylcytosin-N¹-yl, 3.37 g, 22 mmol), dibromoesters 6 and 7 from Example 1 (6.92 g, 24.2 mmol) and potassium carbonate (15.20 g, 110 mmol) was heated to 100°C (bath temperature) with stirring under nitrogen for 3 hours. The mixture was cooled to 50°C and methanol (10 mL) was added with stirring which was continued at 50°C for another hour. The insoluble
30 portion was filtered off using a Celite bed and it was washed with dichloromethane - methanol (4 : 1, 3 × 30 mL). The filtrate was evaporated and the residue was chromatographed on a silica gel column (85 g) using dichloromethane - methanol (9 : 1) as an eluent to give a mixture of methyl esters 11 and 12 (B = cytosin-N¹-yl, R = CH₃, 2.28 g, 46.8% yield) as a pale yellow solid. Their ratio was 7 : 3 as
35 determined by ¹H NMR. Crystallization from ethyl acetate - ethanol (5 : 2) or ethanol afforded pure syn isomer (1.31 g, 26.9% yield).

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Mp. 221 - 223°C.

UV (ethanol) max 295 nm (ϵ 12,500), 230 nm (ϵ 12,000), 205 nm (ϵ 14,700).

IR (KBr) 3360 and 3110 cm^{-1} (NH_2), 1750 cm^{-1} (C=O, ester), 1670, 1635 and 1490 cm^{-1} (cytosine ring and olefin).

5 Calculated for $\text{C}_{10}\text{H}_{11}\text{N}_3\text{O}_3$: C, 54.28; H, 5.01; N, 19.00. Found: C, 54.56; H, 5.25; N, 18.79.

The mother liquor was evaporated and the residue was recrystallized from ethanol to give a mixture of esters 11 and 12 (B = cytosin- N^1 -yl, R = methyl, 550 mg, 11.3% yield) in the ratio of 1 : 2.

10 Mp. 192 - 207°C.

UV (ethanol) max 295 nm (ϵ 12,100), 230 nm (ϵ 11,500), 205 nm (ϵ 14,700).

IR (KBr): 3340 and 3100 cm^{-1} (NH_2), 1750 cm^{-1} (C=O, ester), 1670, 1635 and 1485 cm^{-1} (cytosine ring and olefin).

^1H NMR (CD_3SOCD_3) δ , *syn* isomer 11 (B = cytosin- N^1 -yl, R = methyl) 1.76
15 (ddd, 1) and 1.79 (td, 1, H_4), 2.76 (ddd, 1, H_3), 3.59 (s, 3, OCH_3), 5.81 (d, 1, H_5), 7.35 (s, 1, H_1), 7.44 (s, 2, NH_2), 7.65 (d, 1, H_6); *anti* isomer 12 (B = cytosin- N^1 -yl, R = methyl) 1.92 (ddd, 1) and 2.03 (td, 1, H_4), 2.44 (ddd, 1, H_3), 3.59 (s, 3, OCH_3), 5.84 (d, 1, H_5), 7.44 (s, 3, NH_2 and H_1), 7.95 (d, 1, H_6).

^{13}C NMR (CD_3SOCD_3) ppm, *syn* isomer 11 (B = cytosin- N^1 -yl, R = methyl)
20 9.26 (C_4), 18.30 (C_3), 52.33 (OCH_3), 108.96 (C_2), 116.50 (C_1), 171.32 (C=O); cytosine ring: 96.01 (C_5), 140.30 (C_6), 154.19 (C_4), 165.912 (C_2); *anti* isomer 12 (B = cytosin- N^1 -yl, R = methyl) 12.54 (C_4), 15.50 (C_3), 52.57 (OCH_3), 108.91 (C_2), 117.01 (C_1); cytosine ring: 172.06 (C=O), 96.11 (C_5), 140.48 (C_6), 154.24 (C_4), 165.92 (C_2).

25 EI-MS 221 (M, 16.5), 206 (9.8), 190 (9.8), 162 (100.0), 135 (8.9), 119 (23.1), 111 (15.4).

30 **EXAMPLE 12 - S y n t h e s i s o f s y n - N^1 - (2 - Hydroxymethylcyclopropylidenemethyl)cytosine (1, B = cytosin- N^1 -yl) and *anti*- N^1 -(2-Hydroxymethylcyclopropylidenemethyl)cytosine (2, B = cytosin- N^1 -yl).**

A solution of diisobutylaluminum hydride in cyclohexane (1 M, 35.2 mL, 35.2 mmol) was added dropwise into stirred suspension of 1 : 2 mixture of esters 11 and 12 (B = cytosin- N^1 -yl, R = methyl, 1.73 g, 7.82 mmol) from Example 11 in tetrahydrofuran (230 mL) within 15 min at 0°C under nitrogen. After 4 hours, another
35 portion of diisobutylaluminum hydride (11.7 mL, 11.7 mmol) was added. The stirring was continued at 0°C for another 2 hours. The reaction was quenched by adding

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methanol - water (2:1, 30 mL) dropwise at 0°C. The mixture was then stirred overnight at room temperature. The gel-like solid was filtered off and it was washed with dichloromethane - methanol (1 : 1, 5 × 60 mL) with the aid of a sonicator. The combined filtrate and washings were evaporated to give a mixture of crude products 1 and 2 (B = cytosin-N¹-yl, 1.56 g) as a pale yellow solid.

This mixture (7.82 mmol) was dissolved in refluxing ethanol (200 mL). Benzoic anhydride (1.77 g, 7.82 mmol) was added with stirring into a hot solution and the refluxing was continued for 1 h. Five more portions of benzoic anhydride (1.77 g, 7.82 mmol each) were added every hour. After cooling, the solvent was evaporated to give a pale yellow solid which was partitioned between dichloromethane (150 mL) and saturated aqueous sodium hydrogen carbonate (150 mL). The aqueous phase was extracted with dichloromethane (3 × 50 mL). The combined organic phases were washed with a mixture of saturated aqueous sodium hydrogen carbonate - saturated aqueous sodium chloride (1: 3, 40 mL) and they were dried over sodium sulfate. Removal of the solvents left a pale yellow solid which was chromatographed on a silica gel column using dichloromethane - methanol (98 : 2 and 95 : 5) to give O,N-dibenzoylated compounds (*syn/anti* mixture, 625 mg, 20% yield), N⁴-benzoylated *syn* isomer (1, B = N⁴-benzoylcytosin-N¹-yl, 480 mg, 20.7% yield), and N⁴-benzoylated *anti* isomer (2, B = N⁴-benzoylcytosin-N¹-yl, 960 mg, 41.4% yield). Both isomers were recrystallized from ethanol.

Syn isomer 1 (B = N⁴-benzoylcytosin-N¹-yl):

Mp. 189 - 193°C.

UV (ethanol) max 331 (ε 14,800), 270 nm (ε 19,200), 220 nm (ε 15,400), 206 nm (ε 17,200).

IR (KBr) 3370 cm⁻¹ (OH), 1695, 1665 - 1620, 1560 and 1490 cm⁻¹ (N⁴-benzoylcytosine and olefin), 1050 cm⁻¹ (cyclopropane ring).

¹H NMR (CD₃SOCD₃) δ 1.15 (ddd, 1) and 1.41 (td, 1, H₄), 2.14 (dqd, 1, H₃), 3.31 (dt, overlapped with H₂O at 3.33, 1) and 3.65 (dt, 1, H₅), 5.01 (dd, 1, OH), 7.37 (s, overlapped with H₅, 1, H₁), 7.38 (d, overlapped with H₁, 1, H₅), 8.62 (d, 1, H₆), 11.30 (s, 1, NH), 7.50 (t, 2, meta-H), 7.61 (t, 1, para-H), and 7.99 (d, 2, ortho-H of C₆H₅).

¹³C NMR (CD₃SOCD₃) ppm 5.91 (C₄), 19.44 (C₃), 63.05 (C₅), 115.76 (C₁), 117.51 (C₂); benzoyl group: 128.90 (ortho- and meta-C), 133.23 (para-C) and 133.50

(ipso-C of C₆H₅), 167.78 (C=O); cytosine ring: 97.12 (C₅), 145.33 (C₆), 153.97 (C₄), 163.51 (C₂).

EI-MS 297 (M, 10.4), 280 (15.9), 266 (10.1), 216 (6.7), 176 (6.9), 162 (4.8), 122 (7.3), 105 (100.0).

5 HRMS Calculated for C₁₆H₁₅N₃O₃: M 297.1113. Found: M 297.1108.

Calculated for C₁₆H₁₅N₃O₃: C, 64.62; H, 5.09; N, 14.14. Found: C, 64.50; H, 5.26; N, 14.18.

Anti isomer 2 (B = N⁴-benzoylcytosin-N¹-yl):

Mp. 177 - 181°C.

10 UV (ethanol) max 330 nm (ε 14,600), 269 nm (ε 19,600), 218 nm (ε 14,800), 206 nm (ε 17,200).

IR (KBr) 3300 cm⁻¹ (OH), 1695, 1650, 1620, 1570 and 1485 cm⁻¹ (N⁴-benzoylcytosine and olefin), 1040 cm⁻¹ (cyclopropane ring).

¹H NMR (CD₃SOCD₃) δ 1.36 (ddd, 1) and 1.67 (td, 1, H₄), 1.86 (dqd, 1, H₃),
15 3.38 (dt, 2, H₅), 4.82 (t, 1, OH), 7.44 (d, 1, H₁), 7.41 (d, 1, H₅), 8.46 (d, 1, H₆), 11.30 (s, 1, NH), 7.50 (t, 2, meta-H), 7.61 (t, 1, para-H), and 7.99 (d, 2, ortho-H of C₆H₅).

¹³C NMR (CD₃SOCD₃) ppm 9.03 (C₄), 16.68 (C₃), 63.43 (C₅), 115.60 (C₁),
117.73 (C₂), 128.89 (ortho-C), 128.93 (meta-C), 133.23 (para-C) and 133.49 (ipso-C of C₆H₅), 167.75 (C=O, benzoyl); cytosine ring: 97.35 (C₅), 145.15 (C₆), 154.09 (C₄),
20 163.51 (C₂).

EI-MS 297 (M, 8.6), 280 (18.7), 266 (12.9), 216 (8.4), 176 (3.3), 162 (2.3), 122 (4.9), 105 (100.0);

HRMS Calculated for C₁₆H₁₅N₃O₃: M 297.1113. Found: M 297.1108.

Calculated for C₁₆H₁₅N₃O₃: C, 64.62; H, 5.09; N, 14.14. Found: 64.76; H, 5.04;
25 N, 14.34.

The *syn* isomer 1 (B = N⁴-benzoylcytosin-N¹-yl, 250 mg, 0.84 mmol) was stirred in methanolic ammonia (20%, 30 mL) at room temperature overnight. Evaporation of the resultant solution left a white solid which was recrystallized from ethanol to give *syn* isomer 1 (B = cytosin-N¹-yl, 125 mg, 77% yield).

30 Mp. 222 - 224°C.

UV (ethanol) max 298 nm (ε 13,600), 229 nm (ε 13,500), 204 nm (ε 14,100).

IR (KBr) 3320, 3240, and 3100 cm⁻¹ (OH and NH₂), 1695, 1620 and 1515 cm⁻¹ (cytosine ring and olefin), 1040 cm⁻¹ (cyclopropane ring).

¹H NMR (CD₃SOCD₃) δ 1.01 (ddd, 1) and 1.30 (td, 1, H₄), 2.00 (dqd, 1, H₃),
35 2.80 (dt, overlapped with H₂O at 3.32, 1) and 3.53 (dtd, 1, H₅), 4.93 (dd, 1, OH),

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5.75 (d, 1, H₅), 7.25 (s, 1) and 7.30 (overlapped with H₁, 1, NH₂), 7.30 (q, 1, H₁), 8.13 (d, 1, H₆).

¹³C NMR (CD₃SOCD₃) ppm 5.57 (C₄), 19.28 (C₃), 63.38 (C₅), 111.63 (C₂), 116.06 (C₁); cytosine ring: 95.24 (C₅), 140.95 (C₆), 154.38 (C₄), 165.89 (C₂).

5 EI-MS 193 (M, 10.5), 176 (100.0), 162 (78.5), 149 (4.9), 122 (10.2), 112 (60.9).

HRMS Calculated for C₉H₁₁N₃O₂: M 193.0851. Found: M 193.0854.

Calculated for C₉H₁₁N₃O₂: C, 55.93; H, 5.74; N, 21.76%. Found: C, 55.78; H, 5.94; N, 21.93%.

10 The *anti* isomer 2 (B = N⁴-benzoylcytosin-N¹-yl, 250 mg, 0.84 mmol) was debenzoylated using the procedure described above to give *anti* isomer 2 (B = cytosin-N¹-yl, 132 mg, 81% yield).

Mp. 218 - 220°C.

UV (ethanol) max 296 nm (ε 12,900), 230 nm (ε 12,900), 205 nm (ε 14,900).

15 IR (KBr) 3370, 3310 and 3100 cm⁻¹ (OH and NH₂), 1670, 1630 and 1505 cm⁻¹ (cytosine ring and olefin), 1025 cm⁻¹ (cyclopropane ring).

¹H NMR (CD₃SOCD₃) δ 1.22 (ddd, 1) and 1.56 (td, 1, H₄), 1.74 (dq, 1, H₃), 3.32 (dt, overlapped with H₂O at 3.32, 1, H₅), 4.75 (dd, 1, OH), 5.79 (d, 1, H₅), 7.26 and 7.32 (2s, 2, NH₂), 7.37 (q, 1, H₁), 7.96 (d, 1, H₆).

20 ¹³C NMR (CD₃SOCD₃) δ 8.97 (C₄), 16.15 (C₃), 63.80 (C₅), 111.88 (C₂), 115.87 (C₁); cytosine ring: 95.38 (C₅), 140.69 (C₆), 154.50 (C₄), 165.87 (C₂).

EI-MS 193 (M, 6.7), 176 (100.0), 162 (66.0), 149 (5.5), 122 (13.3), 112 (89.1).

Calculated for C₉H₁₁N₃O₂: C, 55.93; H, 5.74; N, 21.76. Found: C, 55.74; H, 5.92; N, 21.54.

25 **EXAMPLE 13 - Synthesis of Methyl Phenylphosphoro-L-alaninate of *syn*-N⁹-(2-Hydroxymethylcyclopropylidenemethyl)adenine (3, B = adenin-N⁹-yl).**

Phenylmethoxyalaninyl phosphorochloridate (13) in tetrahydrofuran (0.184 M, 25 mL, 4.6 mmol) and N-methylimidazole (0.73 mL, 9.20 mmol) were added to a sonicated suspension of synadenol 1 (B = adenin-N⁹-yl, 200 mg, 0.92 mmol) in pyridine (30 mL) at room temperature. The mixture was then stirred for 3.5 h. The solvents were evaporated and the residue was dried *in vacuo* at room temperature overnight. Chromatography on a silica gel column using gradient dichloromethane - methanol (95 : 5 → 9 : 1) gave the crude product (300 mg) which was

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rechromatographed using dichloromethane - methanol (95 : 5) to furnish compound 3 (B = adenin-N⁹-yl, 270 mg, 64% yield) as a white powder.

Mp 135-149°C.

UV (ethanol) max 277 nm (shoulder, ϵ 9,300), 262 nm (ϵ 13,530), 227 nm (ϵ 28,560), 213 nm (ϵ 28,355).

IR (KBr): 3500 cm⁻¹ (shoulder), 3360 and 3280 cm⁻¹ (shoulder), 3200 and 3140 cm⁻¹ (shoulder, NH and NH₂), 1750 cm⁻¹ (C=O, ester), 1665, 1605 and 1580 cm⁻¹ (adenine and benzene rings, olefin), 1265 and 1245 cm⁻¹ (P=O), 1040, 1010 and 940 cm⁻¹ (phosphoroamidate and cyclopropane).

¹H NMR (CD₃SOCD₃) δ 1.11-1.20 (m, 3, CH₃), 1.34-1.43 (m, 1) and 1.56-1.67 (m, 1, H₄), 2.30-2.44 (m, 1, H₃), 3.51, 3.52 and 3.55 (3s, 3, OCH₃), 3.62-3.88 (m, 1, CH of alanine), 3.90-4.23 (m, 2, H₅), 5.91-6.05 (m, 1, NH of alanine), 7.06-7.17 (m, 3) and 7.24-7.40 (m, 2, phenyl), 7.35 (s, 2, NH₂), 7.41-7.46 (m, 1, H₁), 8.16 and 8.17 (2s, 1, H₂), 8.45, 8.46 and 8.48 (3s, 1, H₈).

¹³C NMR (CD₃SOCD₃): ppm 7.59 (C₄), 17.23, 17.29 and 17.33 (C₃), 68.13 and 68.17 (C₅), 111.92 (C₁), 113.76 and 113.94 (C₂); adenine: 118.87 (C₅), 137.97 (C₈), 148.63 (C₄), 153.51 (C₂), 156.60 (C₆); alanine: 19.96, 20.05 and 20.12 (CH₃), 50.08, 50.12 and 50.21 (CH), 52.25 and 52.29 (OCH₃), 173.56 and 173.85 (C=O); phenyl: 120.56, 120.61 and 120.65 (meta-C), 124.89 (ortho-C), 129.96 (para-C), 151.05 (ipso-C).

³¹P NMR (CD₃SOCD₃) ppm 3.46, 3.64, 3.73 and 3.90.

FAB-MS (thioglycerol matrix) 567 (M + thioglycerol + H, 41.0), 459 (M + H, 11.9), 308 (17.5), 281 (14.2), 234 (21.1), 200 (100.0), 136 (adenine + H, 60.5).

Calculated for C₂₀H₂₃N₆O₅P·0.3H₂O: C, 51.79; H, 5.13; N, 18.12. Found: C, 51.90; H, 5.20; N, 18.14.

EXAMPLE 14 - Synthesis of Methyl Phenylphosphoro-L-alaninate of *anti*-N⁹-(2-Hydroxymethylcyclopropylidenemethyl)adenine (4, B = adenin-N⁹-yl).

The procedure described in Example 13 was used on the same scale starting from *anti*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)adenine (2, B = adenin-N⁹-yl). The reaction mixture was stirred at room temperature for 4 h. After removal of solvents, the residue was dissolved in methanol (1 mL) and it was partitioned between ethyl acetate (250 mL) and water (100 mL). The aqueous phase was extracted once with ethyl acetate (60 mL). The combined organic phases were washed with water (4 × 80 mL) and brine (80 mL). They were dried over Na₂SO₄

and evaporated to leave a syrup. Chromatography on a silica gel column using gradient dichloromethane - methanol (95 : 5 → 92 : 8) gave compound 4 (B = adenin-N⁹-yl) as a white foam (328 mg, 74% yield).

Mp 60-77°C.

- 5 UV (ethanol) max 277 nm (shoulder, ϵ 8,930), 262 nm (ϵ 12,750), 225 nm (ϵ 28,040), 214 nm (shoulder, ϵ 27,210).

IR (KBr) 3330 cm⁻¹ (br) and 3180 (br, NH and NH₂), 1750 cm⁻¹ (C=O, ester), 1645, 1600 and 1580 cm⁻¹ (adenine and benzene rings, olefin), 1250 cm⁻¹ (P=O), 1025 and 937 cm⁻¹ (phosphoroamidate and cyclopropane).

- 10 ¹H NMR (CD₃SOCD₃) δ 1.20 (apparent t, 3, CH₃), 1.52-1.65 (m, 1) and 1.78-1.90 (m, 1, H₄), 2.18 (m, 1, H₃), 3.54, 3.54, 3.56 and 3.58 (4s, 3, OCH₃), 3.77-3.93 (m, 1H, CH of alanine), 3.93-4.10 (m, 2, H₅), 6.01 (apparent d t, 1, NH of alanine), 7.09-7.23 (m, 3) and 7.29-7.45 (m, 2, OC₆H₅), 7.38 (s, 2, NH₂), 7.50-7.56 (m, 1, H₁), 8.18 (s, 1, H₂), 8.48 and 8.48 (2s, 1, H₈).

- 15 ¹³C NMR (CD₃SOCD₃) ppm 10.25 and 10.31 (C₄), 15.32 (C₃), 68.50 (C₅), 111.85 (C₁), 114.36 (C₂); alanine: 20.02 and 20.10 (CH₃), 50.10 and 50.24 (CH of alanine), 52.25 (OCH₃), 174.11 and 174.18 (C=O); adenine: 118.84 (C₅), 137.65 (C₈), 148.71 (C₄), 153.54 (C₂), 156.49 (C₆); phenyl: 120.62 (meta-C), 124.89 (ortho-C), 123.00 (para-C), 151.18 and 151.26 (ipso-C).

- 20 ³¹P NMR (CD₃SOCD₃) ppm 3.55, 3.91 and 3.97.

EI-MS 459 (M + H, 1.5), 458 (M, 6.7), 307 (1.1), 264 (4.1), 200 (100.0), 135 (adenine, 40.8).

HRMS: Calculated for C₂₀H₂₃N₆O₅P: M 458.14675; Found: M 458.1477.

- 25 Calculated for C₂₀H₂₃N₆O₅P·0.4H₂O: C, 51.59; H, 5.15; N, 18.05. Found: C, 51.77; H, 5.26; N, 17.86.

EXAMPLE 15 - Synthesis of Methyl Phenylphosphoro-L-alaninate of *syn*-2,6-diamino-N⁹-(2-Hydroxycyclopropylidene-methyl)purine (3, B = 2,6-diaminopurin-N⁹-yl).

- Phenylmethoxyalaninyl phosphorochloridate (13) in tetrahydrofuran (0.184 M, 30 16.7 mL, 3 mmol) was added dropwise into a suspension of *syn*-2,6-diamino-(2-hydroxymethylcyclopropylidenemethyl)purine (1, B = 2,6-diaminopurin-N⁹-yl, 140 mg, 0.6 mmol) in pyridine (20 mL) with stirring at room temperature. After 5 min., N-methylimidazole (0.48 mL, 6 mmol) was added and the stirring was continued for 3 h. An insoluble gum which formed was dissolved by addition of pyridine (20 mL) 35 and the reaction mixture was stirred overnight at room temperature. The solvents

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were evaporated, the residue was dissolved in 80% acetic acid (35 mL) and the solution was allowed to stand at room temperature for 88 h and then it was evaporated. Chromatography of the crude product on a silica gel column using dichloromethane-methanol (95:5) gave a yellow sirup. This product was dissolved
5 in a mixture of methanol (20 mL) and 0.1 M Na₂HPO₄ (pH 7.5, 20 mL). The solution was stirred overnight at room temperature, it was then evaporated and the product was rechromatographed as shown above. The obtained hygroscopic gum was dissolved in methanol (1 mL), water (10 mL) was added and the resultant suspension was lyophilized to give compound 3 (B = 2,6-diaminopurin-N⁹-yl, 170 mg,
10 60%) as a white solid.

Mp 65-83° C.

UV (EtOH) max 280 nm (ϵ 11,800), 229 nm (ϵ 35,500).

IR (KBr) 3350 and 3200 cm⁻¹ (NH and NH₂), 1745 cm⁻¹ (C=O, ester), 1670-1580 cm⁻¹ (olefin, purine and benzene ring), 1220 cm⁻¹ (P=O), 1160 cm⁻¹ (C-O), 1020
15 and 940 cm⁻¹ (phosphoramidate and cyclopropane).

¹H NMR (CD₃SOCD₃) δ 1.12-1.22 (m, 3, CH₃), 1.27-1.38 (m, 1) and 1.50-1.62 (m, 1, H₄), 2.22-2.40 (m, 1, H₃), 3.52 and 3.55 (2s, 3, OCH₃), 3.68-3.88 (m, 1, Ala-CH), 3.88-4.05 (m, 1) and 4.10-4.30 (m, 1, H₅), 5.91 (s, 2, 2-NH₂), 5.85-6.06 (m, 1, NH), 6.79 (s, 2, 6-NH₂), 7.08-7.20 (m, 3) and 7.20-7.36 (m, 3, H₇ and phenyl), 8.037,
20 8.040, 8.05, and 8.06 (4s, 1, H₈).

¹³C NMR (CD₃SOCD₃) ppm 7.43, 7.50, and 7.605 (C₄), 17.32, 17.41, and 17.80 (C₃), 68.25 and 68.405 (C₅), 111.77 (C₁), 113.26 (C₂); alanine: 19.97, 20.06, and 20.12 (CH₃), 49.94, 50.07, 50.16, and 50.25 (CH), 52.28 (OCH₃), 174.10 and 174.17 (C=O); phenyl: 120.66 (C-meta), 124.93 (C-ortho), 130.00 (C-para), 151.04
25 and 151.14 (C-ipso); purine: 112.16 (C₅), 134.48 and 134.56 (C₈), 150.56 and 150.93 (C₄), 156.60 (C₂), 161.07 (C₆).

³¹P NMR (CD₃SOCD₃) ppm 3.62, 3.85, 3.90 and 4.08.

FAB-MS (thioglycerol matrix) 582 (M + thioglycerol +H, 48.6), 474 (M + H, 11.6), 323 (19.4), 281 (15.7), 249 (27.5), 215 (43.7), 200 (36.2), 151 (2,6-
30 diaminopurine + H, 100.0).

Calculated for C₂₀H₂₄N₇O₅P: C, 50.72; H, 5.11; N, 20.72. Found: C, 50.95; H, 5.06; N, 20.49%.

EXAMPLE 16 - *In Vitro* Antiviral Evaluation Methods

Cells and viruses. The routine growth and passage of KB cells was
35 performed in monolayer cultures using minimal essential medium (MEM) with either

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Hanks salts [MEM(H)] or Earle salts [MEM(E)] supplemented with 10% calf serum. The sodium bicarbonate concentration was varied to meet the buffering capacity required. Cultures of diploid human foreskin fibroblasts (HFF) or MRC-5 cells were grown in medium consisting of MEM(E) with 10% fetal bovine serum. Cells were
5 passed at 1 : 2 to 1 : 10 dilutions according to conventional procedures by using 0.05% trypsin plus 0.02% EDTA in a HEPES buffered salt solution (HBS) (Shipman, C., Jr., *Proc. Soc. Exp. Biol.* 130:305-310 (1969)) as described previously. Turk, S.R., et al., *Antimicrob. Agents Chemother.* 31:544-550 (1987). HFF and MRC-5 cells were passaged only at 1 : 2 dilutions. CEM cells were maintained in
10 suspension culture as detailed previously. Kucera, L.S., et al., *AIDS Res. Human Retroviruses* 9:307-314 (1993).

Virological procedures. Stock HCMV was prepared by infecting HFF cells at a multiplicity of infection (m.o.i.) of <0.01 plaque-forming units (p.f.u.) per cell. Cell growth medium was changed every four days until cytopathology was evident in all
15 cells (approximately 21 days). Supernatant fluids were retained as the virus stock. High titer HSV-1 stocks were prepared by infecting KB cells at an m.o.i. of <0.1 as detailed previously. Turk, S.R., et al., *Antimicrob. Agents Chemother.* 31:544-550 (1987). Virus titers were determined using monolayer cultures of HFF cells for HCMV and monolayer cultures of BSC-1 cells for HSV-1 as described earlier. Prichard,
20 M.N., et al., *J. Virol. Methods* 28:101-106 (1990). Briefly, HFF or BSC-1 cells were planted as described above in 96-well cluster dishes and incubated overnight at 37°C in a humidified 3% CO₂ - 97% air atmosphere. The next day cultures were inoculated with HCMV or HSV-1 and serially diluted 1 : 3 across the remaining eleven columns of the 96-well plate. Cultures were incubated at 37°C for 2 hr to
25 permit virus adsorption and then virus inoculum was replaced with 0.2 mL of fresh medium. Cultures were incubated for seven days for HCMV, two or three days for HSV-1, medium was removed, and the cell sheets were stained with 0.1% crystal violet in 20% methanol. Plaques were enumerated under 20-fold magnification in wells having the dilution which gave 5 to 20 plaques per well. Virus titers were
30 calculated according to the following Formula: Titer (p.f.u./mL) = number of plaques x 5 x 3ⁿ; where n represents the nth dilution of the virus used to infect the well in which plaques were enumerated.

Assays for Antiviral Activity.

(a) HCMV. The effect of compounds on the replication of HCMV has been
35 measured using a plaque reduction assay. HFF cells in 24-well cluster dishes were

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infected with approximately 100 p.f.u. of HCMV per cm² cell sheet using the procedures detailed above. Following virus adsorption, compounds dissolved in growth medium were added to duplicate wells in three to six selected concentrations. Following incubation at 37°C for 7 to 10 days, cell sheets were fixed, stained with crystal violet and microscopic plaques enumerated as described above. Drug effects were calculated as a percentage of reduction in number of plaques in the presence of each drug concentration compared to the number observed in the absence of drug. Ganciclovir (DHPG) was used as a positive control in all experiments.

The effect of compounds on the replication of HCMV also was measured using a yield reduction assay. HFF cells were planted as described above in 96-well cluster dishes, incubated overnight, medium removed and the cultures were inoculated with HCMV at a m.o.i. of 0.5 to 1 p.f.u. per cell as reported elsewhere. After virus adsorption, inoculum was replaced with 0.2 mL of fresh medium containing test compounds. The first row of 12 wells was left undisturbed and served as virus controls. Each well in the second row received an additional 0.1 mL of medium with test compound at three times the desired final concentration. The contents of the 12 wells were mixed by repeated pipetting and then serially diluted 1 : 3 along the remaining wells. In this manner, six compounds could be tested in duplicate on a single plate with concentrations from 100 μ M to 0.14 μ M. Plates were incubated at 37°C for seven days, subjected to one cycle of freezing and thawing; aliquots from each of the eight wells of a given column were transferred to the first column of a fresh 96-well monolayer culture of HFF cells. Contents were mixed and serially diluted 1 : 3 across the remaining eleven columns of the secondary plate. Each column of the original primary plate was diluted across a separate plate in this manner. Cultures were incubated, plaques were enumerated, and titers calculated as described above.

(b) HSV-1. An enzyme-linked immunosorbent assay (ELISA) was employed to detect HSV-1. 96-well cluster dishes were planted with BSC-1 cells at 10,000 cells per well, in a total volume of 200 μ L per well of MEM(E) plus 10% calf serum. After overnight incubation at 37°C, drug and HSV-1 was added at the rate of 100 PFU/well. ELISA plates were blocked with 200 μ L per well of 10% calf serum and 0.05% tween in HBS. After incubation for 30 minutes, the blocking agent was rinsed two times with HBS-T. A 1 : 400 dilution of AP conjugated rabbit anti-HSV-1 antibody in HBS-F was added. Plates were sealed with adhesive sheet, and incubated on rocker for one hour at 37°C. Plates were developed in the dark with 100 μ L per well

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of substrate solution containing p-nitrophenyl phosphate. Plates were read at 492 nm. Drug effects were calculated as a percentage of the reduction in virus in the presence of each drug concentration compared to the titer obtained in the absence of drug. Acyclovir was used as a positive control in all experiments.

- 5 (c) HHV-6. In this case, enzyme-linked immunosorbent assay (ELISA) was performed in covalent amine plates (Costar, Cambridge, MA). The plates were activated by the addition of a homobifunctional crosslinking agent, bis(sulfosuccinimidyl) suberate, which was dissolved at 1 mg/mL in 30 mL of phosphate buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.4 mM KH₂PO₄, pH 7.4) and 300 μ L of the crosslinker was added to each well in the
10 covalent plate. The crosslinker reacted with the amine function on the plate for 30 min at room temperature. The byproduct, sodium N-hydroxysuccinimide sulfite, was removed by decanting and washing the plate twice with PBS. Samples consisting of 150 μ l of mixed suspended HSB₂ cells from the original drug-treated plate were
15 solubilized in an equal volume of 10% Triton X-100 in coating buffer (15 mM Na₂CO₃, 3.5 mM NaHCO₃, pH 9.6). The plate was covered and then incubated for 1 h at 37°C in a 5% CO₂ atmosphere. These binding conditions facilitated covalent attachment of the antigen to the free end of the crosslinker.

- After covalent binding, the antigen solution was decanted and the plate was
20 washed six times in HEPES buffered saline (Shipman, C., Jr., *Proc. Soc. Exp. Biol.* 130:305-310 (1969)) with 0.05% Tween 20 (HBS-T), soaking for three min for each wash. Unbound sites on the plate were blocked with 300 μ L per well of 2% lowfat dry milk in PBS (blocker) for 30 min at room temperature on a shaker. The blocker was decanted and 50 μ L of the diluted primary monoclonal antibody, specific for
25 HHV-6 (GS) glycoprotein gp116, was added. The antibody solution consisted of antibody diluted 1 : 400 in equal volumes of blocker and 10% Triton X-100 in coating buffer. The presence of both blocker and detergent in the antibody solutions was necessary to reduce background signal. The plate was then covered and incubated for 1 h at 37°C. The plate was washed again, as described above, then blocker was
30 added again, as before. Next, each well received 100 μ L of a solution of the secondary antibody, horse radish peroxidase-labeled rabbit anti-mouse antibody, diluted to 1 : 400 (as above). The plate was incubated for 1 h at 37°C. The plate was washed again as described above, and developed using 100 μ L/well of TMB-Turbo (Pierce, Rockford, IL) for 30 min, at room temperature. The reaction was

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stopped with 50 μ L/well 2 M H₂SO₄. Absorbance in each well was determined at 450/570 nm.

(d) HIV-1. Reverse transcriptase (RT) was employed as a marker for HIV-1. This assay measured the presence of HIV in supernatants of CEM cells infected with strain III_B of HIV-1 by the amount of RT activity. Cells were grown, infected, and incubated in the presence of seven concentrations (one-half log₁₀ dilutions) beginning at 1 or 100 μ M of compounds to be assayed. Procedures and the RT assay were performed as detailed previously. Kucera, L.S., et al., *AIDS Res. Human Retroviruses* 9:307-314 (1993); White, E.L., et al., *Antiviral Res.* 16:257-266 (1991).

Cytotoxicity assays. Two different assays were used to explore cytotoxicity of selected compounds as we have detailed previously. (i) Cytotoxicity produced in stationary HFF cells and in growing CEM cells was determined by microscopic inspection of cells used in plaque and RT assays which were not affected by the virus. Turk, S.R., et al., *Antimicrob. Agents Chemother.* 31:544-550 (1987). (ii) The effect of compounds during two population doublings of KB cells was determined by crystal violet staining and spectrophotometric quantitation of dye eluted from stained cells. Prichard, M.N., et al., *Antimicrob. Agents Chemother.* 35:1060-1065 (1991).

Data Analysis. Dose-response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentrations. Fifty-percent inhibitory (IC₅₀) concentrations were calculated from the regression lines. Samples containing positive controls (acyclovir for HSV-1, ganciclovir for HCMV, and 2-acetylpyridine thiosemicarbazone for cytotoxicity) were used in all assays.

Testing Results. Compounds of Formula 1 exhibit a significant activity against herpes viruses. It was found that compounds of the present invention strongly inhibit the replication of HCMV as measured by plaque and yield reduction assays using HFF as host cells by the method described above and they also inhibit the replication of HSV-1 as determined by enzyme-linked immunosorbent assay (ELISA).

	Compound	IC ₅₀ (μ M)		
		HCMV ^a	HCMV ^b	HSV-1 ^c
	1 (B=adenine), Example 5	2.0	1.5	3.7
	1 (B=guanine), Example 8	2.0	1.8	100
5	1 (B=cytosine), Example 12	28	16	>100
	1 (B=2,6-diaminopurine), Example 9	15	18	>100
	1 (B=2-amino-6-cyclopropylaminopurine), Example 10	0.4	1.0	20
10	1 (B=2-amino-6-chloropurine), Example 7	5.0	0.8	40
	3 (B=adenine), Example 13	0.14	0.21	2.5
	4 (B=adenine), Example 14	21	—	65
15	3 (B=diaminopurine), Example 15	4	—	50

^aPlaque reduction.^bYield reduction, IC₅₀.^cELISA.

These effects are comparable with those of ganciclovir, current drug of choice for HCMV.

Compounds of the present invention also strongly inhibit HHV 6 to a greater extent than current drug foscarnet (Foscavir) as determined by enzyme-linked immunosorbent assay (ELISA) in HSB-2 cells by the method described above.

Compound	HHV-6, IC ₅₀ (μ M)
1 (B=adenine), Example 5	3.0
Foscarnet	15.0

Compounds of the present invention also inhibit HIV-1 when tested in a reverse transcriptase assay as described above.

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Compound	HIV-1, IC ₅₀ (μM)
1 (B=guanine), Example 8	1.3
3 (B=adenine), Example 13	<0.14
4 (B=adenine), Example 14	1.6
3 (B=2,6-diaminopurine), Example 15	0.26

In the cell line used to propagate HIV-1, no visual toxicity was observed at 100 μM.

Compounds of the present invention were tested for cytotoxicity in a culture of HFF and KB cells according to the methods described above.

Compounds	IC ₅₀ (μM)		
	HFF (visual)	KB (growth)	CEM (visual)
1 (B=adenine), Example 5	>100	82	>100
1 (B=guanine), Example 8	>100	>100	>100
1 (B=cytosine), Example 12	>100	>100	>100
1 (B=2,6-diaminopurine), Example 9	>100	>100	>100
1 (B=2-amino-6-cyclopropylaminopurine), Example 10	>100	>100	>100
1 (B=2-amino-6-chloropurine), Example 7	>100	>100	—
3 (B=adenine), Example 13	2.5	0.5	1.1
4 (B=adenine), Example 14	100	50	66
3 (B=2,6-diaminopurine), Example 15	>100	40	100

EXAMPLE 17 - *In vivo* Antiviral Evaluation Method

Compounds 1 (B = adenin-N⁹-yl and guanin-N⁹-yl) were evaluated in mice infected with murine cytomegalovirus (MCMV). Mice were infected intraperitoneally with sufficient MCMV to produce 90 to 100% mortality. Compounds were administered once daily for five days at doses of 5.6, 16.7 and 50 mg/kg by intraperitoneal injection in a 0.4% suspension of carboxymethylcellulose. Ganciclovir was administered in sterile water at the same doses as a positive control drug. All

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compounds were administered to separate groups of 15 mice each at 6, 24, and 48 hours after infection with MCMV. Groups of 10 uninfected mice also received test compounds as toxicity controls at the doses listed above.

Compound 1 (B = adenin-N⁹-yl) reduced mortality from 100% in placebo treated mice to 33% in mice treated with 50 mg/kg of the drug. Decreasing effects were noted at lower doses and when drug administration was delayed until 24 or 48 hours after infection. No deaths were observed in uninfected mice that received only drug.

In a second experiment, compound 1 (B = guanin-N⁹-yl) reduced mortality from 90% in placebo treated mice to about 7 to about 13% in mice treated with 16.7 or 50 mg/kg of this drug. No deaths were observed in uninfected mice that received only drug. In a similar manner, ganciclovir reduced mortality to about 0 to about 20% at these doses and it was somewhat more effective than compound 1 (B=guanin-N⁹-yl) at 5.6 mg/kg.

A compound selected from the group consisting of *syn*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)adenine, *syn*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)guanine, *syn*-2,6-diamino-N⁹-(2-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine, *syn*-2-amino-6-cyclopropylamino-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine, *syn-syn*-2-amino-6-chloro-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine, *syn*-N¹-(2-hydroxymethylcyclopropylidenemethyl)cytosine and pharmaceutically acceptable salts thereof. A pharmaceutical composition comprising the compounds listed above and pharmaceutically acceptable salts thereof. Prodrugs of the compounds listed above and pharmaceutically acceptable salts thereof. A method of treatment of mammals infected with a virus comprising administering an effective amount of a compound listed above. The method above wherein said mammal is a human and said virus is a human herpes virus. The method above wherein said mammal is a human and said virus is human immunodeficiency virus. The method above wherein said mammal is a human and said virus is hepatitis B virus. The method above wherein the compounds listed above are used in combination with other antiviral drugs such as acyclovir, ganciclovir and zidovudine.

Those skilled in the art can now appreciate from the foregoing description that the broad teachings of the present invention can be implemented in a variety of forms. Therefore, while this invention has been described in connection with particular examples thereof, the true scope of the invention should not be so limited

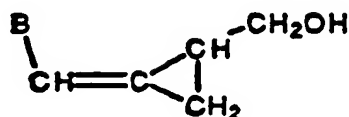
- 38 -

since other modifications will become apparent to the skilled practitioner upon a study of the drawings and specification.

All patents and other publications cited herein are expressly incorporated by reference.

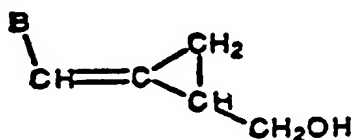
WE CLAIM:

1. A compound having the formula:



wherein B is a heterocyclic ring derived from a purine or pyrimidine moiety, and pharmaceutically acceptable salts, and prodrugs, thereof.

- 5 2. A compound having the formula:



wherein B is a heterocyclic ring derived from a purine or pyrimidine moiety, and pharmaceutically acceptable salts, and prodrugs, thereof.

3. The compound of Claims 1 or 2, wherein B is selected from the group consisting of 6-aminopurine, 2,6-diaminopurine, 2-amino-6-cyclopropylaminopurine, 6-hydroxypurine, 2-amino-6-halo substituted purine, 2-amino-6-alkoxy substituted purine, 2-amino-6-hydroxypurine, 3-deazapurine, 7-deazapurine, 8-azapurine, cytosine, 5-halo substituted cytosine, 5-alkyl substituted cytosine, thymine, uracil and 6-azapyrimidine.

4. The compound of Claims 1 or 2, wherein B is selected from the group consisting of adenin-N⁹-yl, guanin-N⁹-yl, cytosin-N¹-yl, 2,6-diaminopurine, 2-amino-6-cyclopropylaminopurin-N⁹-yl and 2-amino-6-chloropurin-N⁹-yl.

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5. An antiviral compound selected from the group consisting of *syn*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)adenine, *syn*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)guanine, *syn*-N¹-(2-hydroxymethylcyclopropylidenemethyl)cytosine, *syn*-2,6-diamino-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine, *syn*-2-amino-6-cyclopropylamino-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine, *syn*-2-amino-6-chloro-N⁹-(2-hydroxymethylcyclopropylidenemethyl)purine and pharmaceutically acceptable salts, and prodrugs, thereof.

6. An antiviral compound selected from the group consisting of methyl phenyl-phosphoro-L-alaninate of *syn*-N⁹-(2-hydroxymethylcyclopropylidenemethyl)adenine, methyl phenyl-phosphoro-L-alaninate of *anti*-N²-(2-hydroxymethyl-cyclopropylidenemethyl)adenine and pharmaceutically acceptable salts, and prodrugs, thereof.

7. A composition comprising a compound of Claims 1-6 and a pharmaceutically acceptable carrier.

8. A method of treating mammals infected with a virus comprising the step of administering to the mammal an antiviral compound selected from the group consisting of the compounds of Claims 1-6, and combinations thereof.

9. The method of Claim 8, wherein said mammal is a human.

10. The method of Claim 8, wherein said virus is a human herpes virus.

11. The method of Claim 8, wherein said virus is a human immunodeficiency virus.

12. The method of Claim 8, wherein said virus is hepatitis B virus.

13. The method of Claim 8, further comprising the step of administering an additional antiviral compound.

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14. The method of Claim 13, wherein the additional antiviral compound is selected from the group consisting of acyclovir, ganciclovir, zidovudine, AZT, ddl, ddC, d4T, and combinations thereof.

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FIGURE 1

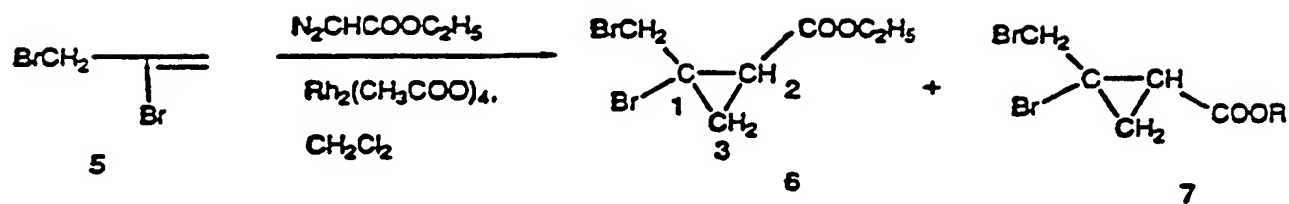
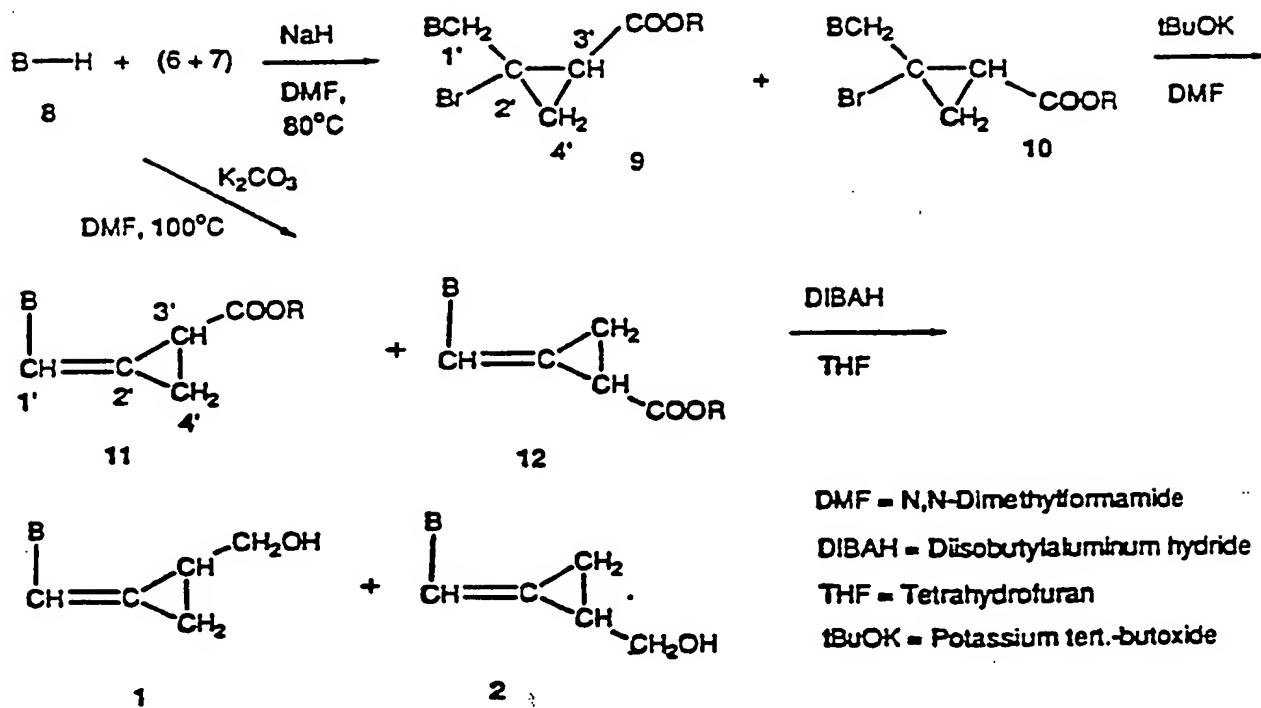
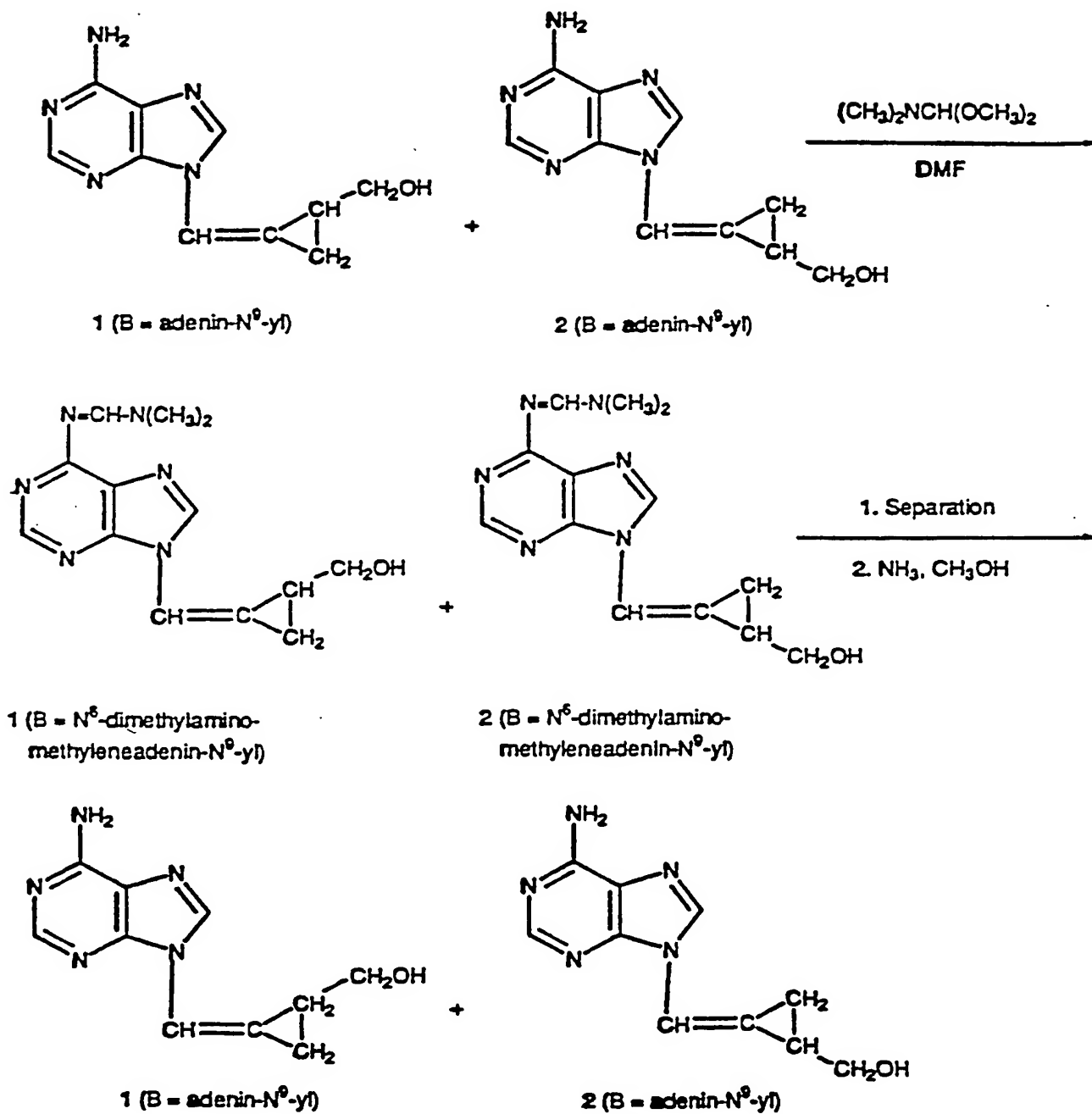


FIGURE 2



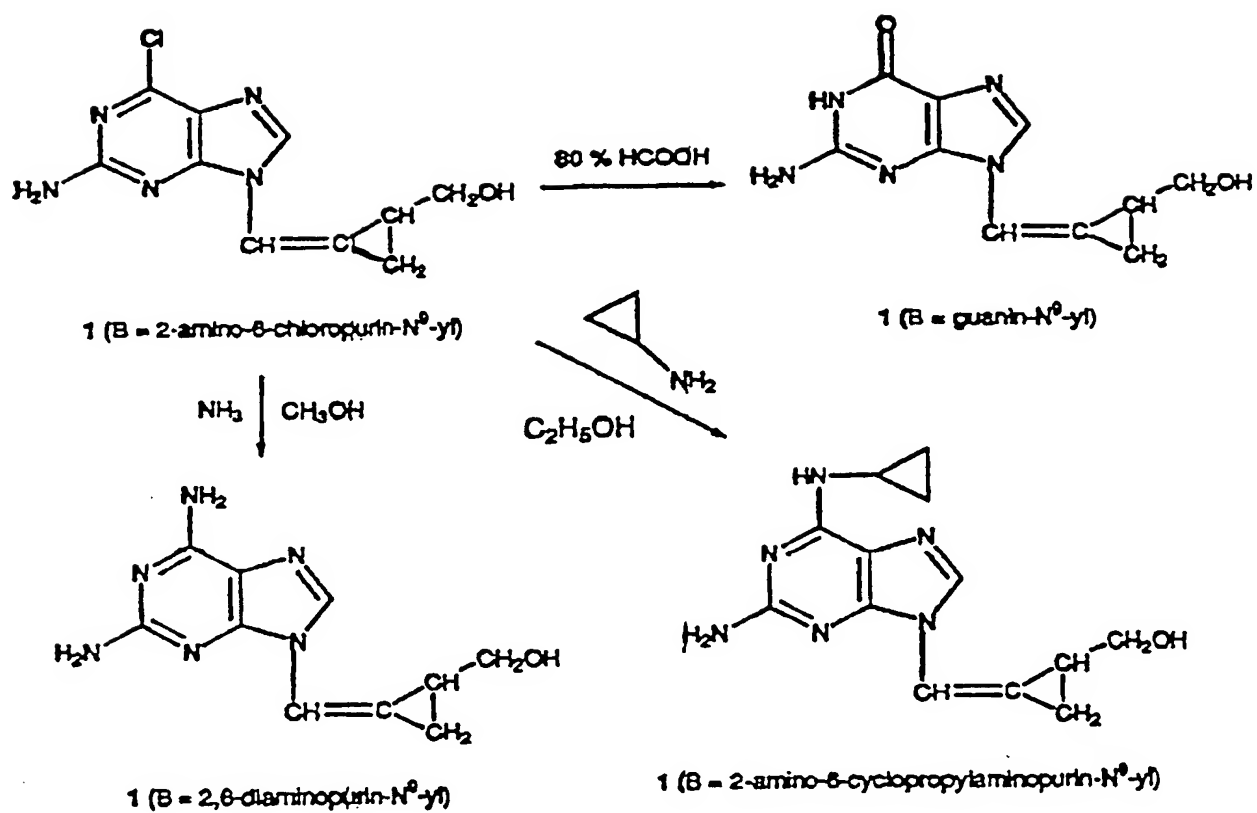
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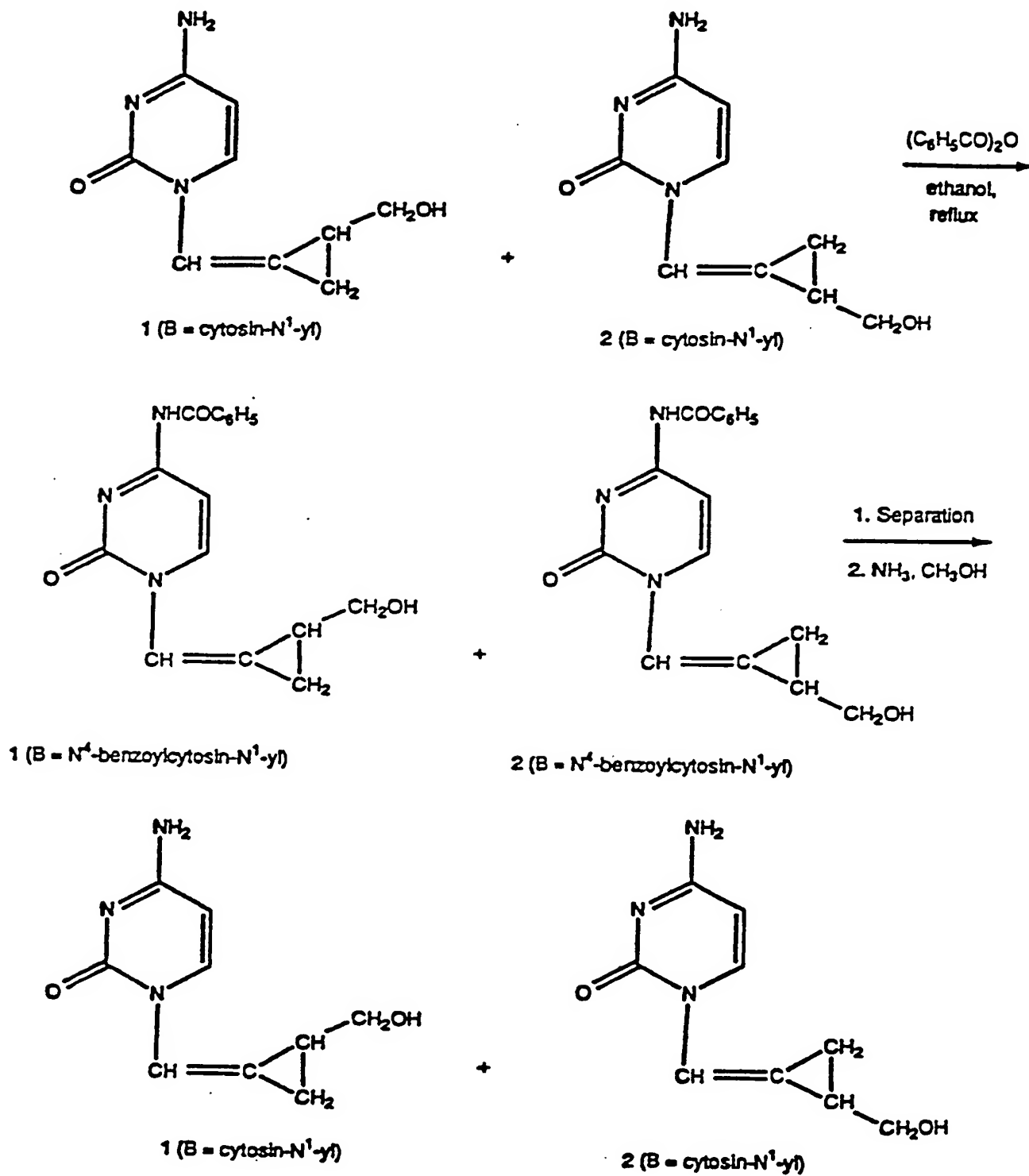
FIGURE 3



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FIGURE 4



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FIGURE 5

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FIGURE 6

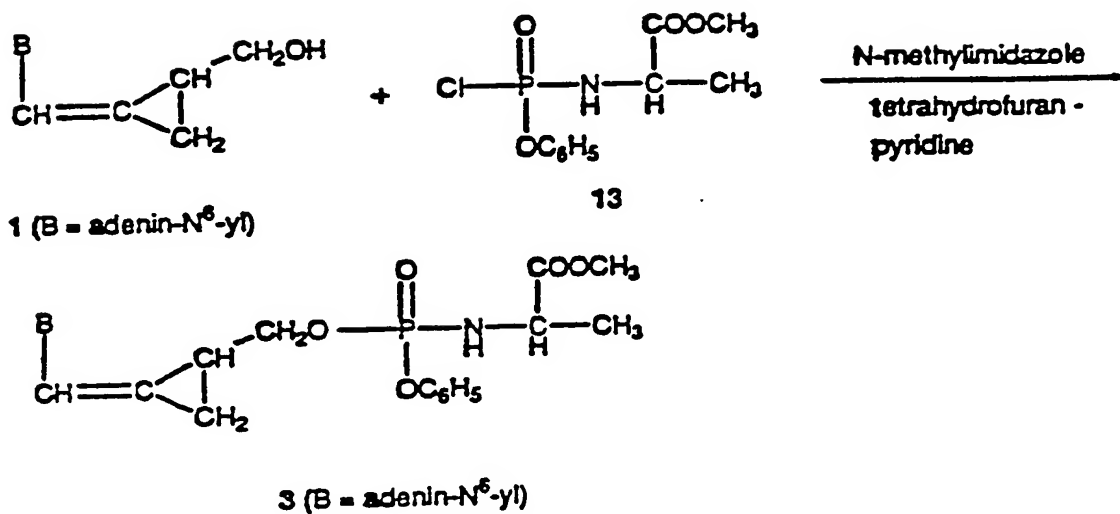
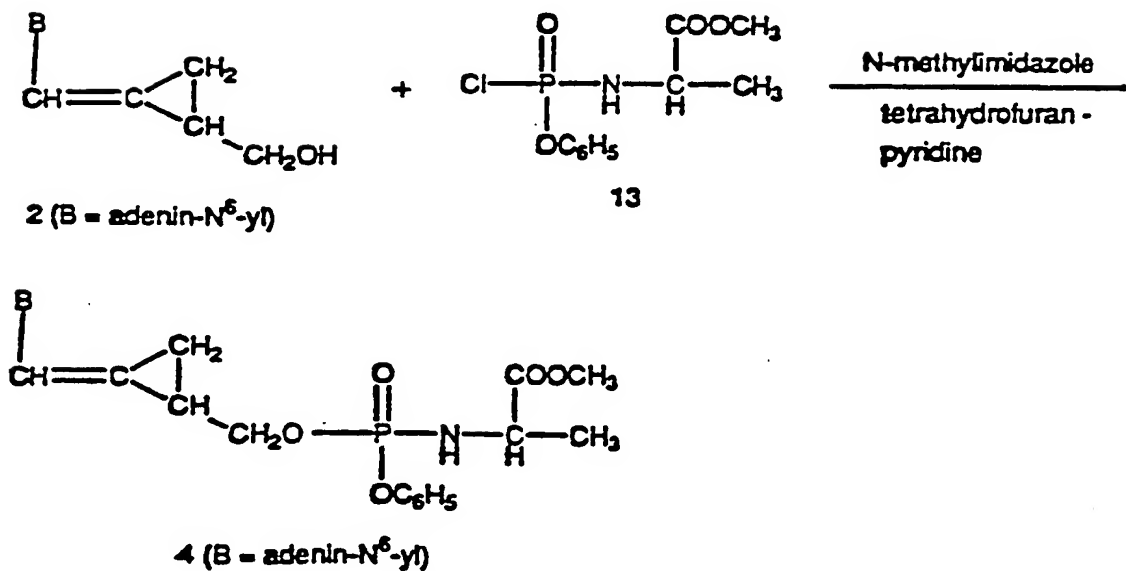


FIGURE 7



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/00440

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS Online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Qui et al. (Z)- and (E)-2-((Hydroxymethyl) cyclopropylidene) methyladenine and -guanine. New Nucleoside Analogs with a Broad-Spectrum Antiviral Activity. J. Med. Chem. January 1998 Vol. 41(1), 10-23. See structures 14-17, 28, 29, 33, 34.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

09 APRIL 1998

Date of mailing of the international search report

16 JUN 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Washington, D.C. 20231

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MARK L. BERCH aco

Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/00440

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/00440

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07D 473/16, 473/18, 473/30, 473/34, 239/47, 239/54, 239/553, 487/04, 471/04 A61K 31/52, 31/505, 31/44, 31/53, 31/675; C07F 9/6561, 9/6521, 9/6512

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

544/243, 244, 276, 277, 280, 265, 182; 546/23, 118
514/261, 262, 274, 81, 84, 86

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

544/243, 244, 276, 277, 280, 265, 182;
514/261, 262, 274, 81, 84, 86
546/23, 118

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I Purines - Claims 1-5 (part), 6, 7-14 (part).

Group II Pyrimidines - Claims 1-5 (part), 7-14 (part).

Group III Other (aza-and deaza-purines; aza pyrimidines) Claims 1-3 (part), 7-14 (part).

The inventions listed as Groups I, II, III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Each has a special technical feature lacking in the other two. Group I has a purine as the nucleoside base. Group II has a pyrimidine. Group II has a triazine, pyridomimidazole, etc. Thus, the special technical feature essential for the pharmaceutical activity, differs in each group.